Decreased Myocardial Perfusion Reserve in Diabetic Autonomic Neuropathy

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The pathophysiological mechanisms responsible for increased cardiovascular mortality in diabetic autonomic neuropathy are unknown. To investigate the effect of autonomic neuropathy on myocardial function, we performed dynamic contrast-enhanced magnetic resonance perfusion imaging during baseline conditions and after Dipyridamole-induced vasodilatation in nine type 1 diabetic patients with autonomic neuropathy (AN+), defined by cardiovascular tests, as well as in 10 type 1 diabetic patients without autonomic neuropathy (AN−) and 10 healthy control subjects. Baseline myocardial perfusion index (K_i) was similar in the three groups (AN+ 88.6 ± 8.7 ml · 100 g^{-1} · min^{-1}, AN− 82.6 ± 7.2, control subjects 93.7 ± 9.0) (means ± SE). K_i during Dipyridamole vasodilatation was significantly lower in the patients with autonomic neuropathy ($P < 0.001$) than in the other groups (AN+ 131.1 ± 13.0 ml · 100 g^{-1} · min^{-1}, AN− 177.3 ± 8.6, control subjects 197.2 ± 8.9). Mean blood pressure was unchanged during Dipyridamole infusion in AN− and control subjects, whereas a significant blood pressure decrease was found in AN+ (15.6 ± 2.6 mmHg, $P < 0.025$). There was a significant correlation between blood pressure response to Dipyridamole and myocardial perfusion reserve index. We conclude that type 1 diabetic patients with autonomic neuropathy have a decreased myocardial perfusion reserve capacity when challenged with a vasodilatator, a finding that may in part be the pathophysiological substrate for the increase in mortality in these patients. The underlying mechanism may be defective myocardial sympathetic vasodilatation, a lack of ability to maintain blood pressure during vasodilatation, or both. *Diabetes* 51:3306–3310, 2002

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ECG, electrocardiogram; K_i, myocardial perfusion index; LVMI, left ventricular mass index; MIBG, [^{123}I]-metaiodobenzylguanidine; MR, magnetic resonance; MRI, MR imaging; PET, positron emission tomography; ROI, region of interest; SPECT, single-photon emission tomography.

Autonomic neuropathy is a frequent complication in diabetic patients (1). It interferes with the physiological adaptation to everyday activities, causing considerable morbidity (2), and the mortality has been reported to be increased in diabetic autonomic neuropathy (3,4). The excess mortality is explained, to some extent, by coexistence of diabetic neuropathy and macrovascular disease, but these conditions cannot fully account for the increase in mortality (5). The pathophysiological mechanisms underlying the grave prognosis have not been identified, although many studies have focused on cardiovascular function, since diabetic patients with autonomic neuropathy often face sudden cardiac death (6,7). Much attention has been paid to the potential increase in arrythmogenicity (8–10), and few studies have focused on myocardial perfusion (11,12). Dynamic contrast-enhanced magnetic resonance (MR) perfusion imaging allows noninvasive estimation of myocardial perfusion (or a perfusion index expressed as the unidirectional constant K_i) during baseline conditions and during maximal hyperemia induced with Dipyridamole (13–16). The aim of the present study was to measure these parameters in diabetic patients with and without autonomic neuropathy, the two groups that have microvascular disease (nephropathy and retinopathy) to a similar and limited extent.

RESEARCH DESIGN AND METHODS

Study population. Nineteen type 1 diabetic patients and 10 healthy control subjects volunteered for the study after giving written informed consent (Table 1). The study was approved by the Local Ethics Committee for Copenhagen and Frederiksberg Municipality (J.nr. KF 01-390/98). All type 1 diabetic patients were C-peptide negative, and none of the patients had major microvascular complications (patients were selected from those at the outpatient clinic at Hvidovre Hospital who did not have proliferative retinopathy and/or nephropathy). The patients were divided into two groups according to the outcome of three autonomic nerve function tests: heart rate variation during deep breathing, Valsalva ratio, and blood pressure responses to standing up. The patients were classified as having autonomic neuropathy if two of these three tests were abnormal (17,18) (Table 2). In addition, somatic nerve function (vibratory perception threshold) was evaluated by Bio-Thesiometry (Biomedical Instruments, Newbury, OH). Thus, the two patient groups had markedly different autonomic and somatic nerve function but did not differ significantly in other parameters (age, duration of diabetes, BMI, presence of microalbuminuria and retinopathy, and daily insulin dose). The patients had no signs or symptoms of any disease other than diabetes. They had no cardiac disease, as reflected by a normal electrocardiogram (ECG) and a normal echocardiography, including assessment of wall thickness, ejection fraction, wall motion, and valvular function. Apart from insulin, the patients took no medications.

Protocol. The patients and the healthy subjects met in the MR laboratory on two separate days.

TABLE 1
Anthropometric data

	AN+	AN−	Control subjects
n	9	10	10
Age (years)	49 ± 2	44 ± 2	47 ± 1
Duration of diabetes (years)	22 ± 4	22 ± 4	—
Height (cm)	177 ± 3	169 ± 4	174 ± 2
Weight (kg)	76 ± 5	67 ± 4	75 ± 3
Daily insulin dose (IU)	47 ± 6	39 ± 2	0
HbA_{1c} (%)	8.8 ± 0.5	8.8 ± 0.4	5.8 ± 0.2

Data are means ± SE.

Day 1: measurement of myocardial perfusion during baseline conditions. The subjects met in the laboratory at least 4 h after breakfast, with the diabetic patients having taken their normal dose of insulin before breakfast. Subjects were told to abstain from consuming coffee, tea, chocolate, and tobacco at least 24 h before study day 1. They were placed in the supine position in the MR scanner, and a cannula was inserted into a cubital vein. After 30 min of supine rest, contrast agent (0.08 mg/kg Gadolinium-DTPA) was injected intravenously and MR imaging (MRI) was performed during baseline conditions. The MR scanning was completed in 30 min.

Day 2: measurement of myocardial perfusion during Dipyridamole-induced vasodilatation. The protocol was identical to day 1, except that the subjects had 0.56 mg/kg Dipyridamole injected intravenously over a period of 4 min. Eight minutes after intravenous Dipyridamole, the perfusion measurements were initiated.

The sequence was randomized with an interval of no more than 7 days between the experiments. Blood glucose was measured immediately before the experiments to document the absence of hypoglycemia. Heart rate, ECG, and blood pressure were monitored continuously during the experiments, using three precordial electrodes and a Dinamap XL (Kivex A/S, Copenhagen) apparatus for blood pressure registration.

MRI

Imaging protocol. The study was performed using a 1.5 T Siemens Vision MR scanner, using a body phase array coil placed corresponding to the thorax. A standard inversion recovery turbo FLASH sequence (IR-turboFLASH) was modified to obtain five slices, acquiring one slice at a time (19). In this study, only the first slice was analyzed, because we anticipated a nonfocal perfusion abnormality, if any. The initial 180° resonant frequency pulse was a nonselective adiabatic pulse. The first slice was placed nearest to the base of the heart and had a TI of 15 ms. The time of repetition was 2.4 ms and the echo time was 1.2 ms (field of view 300 mm with 80 phase-encoding steps and 128 readout point, i.e., matrix size 80 × 128). The center of k-space was placed symmetrically in both directions. Each phase-encoding step was generated with a flip angle of 15°. Slice thickness was 10 mm.

After placing the patient in the magnet, several scout images of the heart were obtained to image the bolus passage in the short axis plane. Preceding the contrast agent injection, T1 was measured in every slice using the inversion recovery turbo FLASH sequence, by varying TI. Ten values of TI were chosen, ranging from 15 to 4,000 ms. The bolus passage was followed by 100 consecutive frames, triggered on every third *R*-wave in the ECG, giving a time resolution of ~3 s. Receiver gain was kept constant during the entire imaging. The contrast agent was injected via cubital vein using a power injector (0.08 mmol/kg, speed 3 ml/s) (Magnevist; Schering) after acquiring the first 10 sets of images.

Calculation and image analysis. All images were transferred to a separate computer and evaluated in MATLAB. Region of interest (ROI) was placed corresponding to the septal, anterior, lateral, and posterior wall of the left ventricle. The ROIs were placed both corresponding to the T1 measurement and corresponding to the 100 frames during the bolus passage. All ROIs were checked and moved accordingly if movement of the heart occurred due to respiration. A detailed description of the calculation can be found elsewhere (19); the following is a short description: corresponding to the T1 measurement, the signal from the ROIs was read out and the relevant MR signal equation was fitted to estimate T1 (=1/R1) and M0. The concentration in tissue and blood was calculated and based on the linear relation between change in R1 and concentration and formulated as $\Delta R1 = r1C$, where r1 is the relaxivity (liters per moles per second), taken from the literature. An inherent advantage of this method is the elimination of the inhomogenity in coil sensitivity. K_i and volume of distribution (8) were then calculated for each ROI by a normal deconvolution procedure using a simple exponential kernel. K_i is related to the perfusion (F) and the extraction fraction (E) of the contrast agent as $K_i = EF$. The usefulness of K_i has previously been validated in a number of studies of patients with ischemic heart disease (13,16).

Statistical analysis. Data are given as means ± SE. Differences in mean values between the groups were tested using the *t* test for unpaired comparisons, and linear regression analysis was performed using standard methodology. Variation between groups was tested using ANOVA. All analyses were performed with the SAS computer program. A *P* value (two tailed) <0.05 was considered significant.

RESULTS

Myocardial perfusion was evaluated in four ROIs: anterior, posterior, lateral, and septal. Neither during baseline conditions nor during vasodilatation with Dipyridamole did K_i differ within the four ROIs in the individual groups (Table 3). Accordingly, overall K_i values (mean values from four ROIs) are given in Fig. 1. There were no significant differences in baseline K_i among the three groups. Left ventricular mass index (LVMI [left ventricular mass/body surface area]) did not differ significantly among the three groups (type 1 diabetic patients with autonomic neuropathy [AN+] 92 ± 6 $g \cdot m^{-2}$, type 1 diabetic patients without autonomic neuropathy [AN−] 86 ± 4, control subjects 87 ± 7) (means ± SE). However Dipyridamole-induced vasodilatation response was significantly attenuated ($P < 0.001$) in AN+ compared with AN− and control subjects (AN+: baseline 88.6 ± 8.7 ml · 100 g^{-1} · min^{-1} vs. Dipyridamole 131.1 ± 11.0; AN−: 82.6 ± 7.2 vs. 177.3 ± 8.6; control subjects: 93.7 ± 9.0 vs. 197.2 ± 8.9). The increase in K_i to Dipyridamole did not differ significantly between AN− and control subjects (Fig. 1). Heart rate increased significantly ($P < 0.01$) in AN− and control subjects in response to Dipyridamole infusion (Fig. 2), whereas no significant increase was found in AN+. Mean blood pressure did not change significantly in AN− and control subjects, whereas a significant decrease occurred ($P < 0.025$) in AN+ (Fig. 2). Stepwise multiple regression analysis was performed to determine independent predictors of myocardial perfusion response to Dipyridamole, including age, sex, duration of diabetes, urinary albumin/

TABLE 2
Neuropathy status, nephropathy, and retinopathy

	AN+	AN−	Control subjects
n	9	10	10
Beat-to-beat variation (min^{-1})	2 ± 0.7	18 ± 2	17 ± 2
Valsalva ratio	1.05 ± 0.02	1.21 ± 0.05	1.29 ± 0.01
Postural blood pressure decrease (mmHg)	26 ± 8	14.4 ± 3	9 ± 1
Vibration perception threshold (mV)	34 ± 5	11 ± 2	—
Urinary albumin excretion (mg/12 h)	83 ± 15	10 ± 3	6 ± 0.7
Simplex retinopathy (*n*)	2	0	—

Data are means ± SE.

TABLE 3
Regional myocardial blood flow before and during Dipyridamole infusion

	Anterior		Lateral		Posterior		Septal	
	Before	During	Before	During	Before	During	Before	During
AN+ (n = 9)	86.5 ± 5.0	125.5 ± 14.2	90.7 ± 7.1	121.7 ± 11.6	88.7 ± 9.0	130.3 ± 15.5	99.0 ± 7.9	130.5 ± 13.1
AN− (n = 10)	86.6 ± 6.9	174.2 ± 7.6	77.3 ± 7.0	173.4 ± 8.7	83.1 ± 6.8	180.0 ± 9.0	83.2 ± 5.6	178.2 ± 10.0
Control subjects (n = 10)	93.4 ± 4.8	200.1 ± 12.9	93.0 ± 6.2	191.3 ± 7.3	91.3 ± 6.7	199.3 ± 11.3	97.1 ± 6.4	204.0 ± 6.8

Data are means ± SE.

creatinine ratio, LVMI, HbA_{1c}, and heart rate and blood pressure response to Dipyridamole. In the final model, the only significant predictor of the perfusion response was the blood pressure response to Dipyridamole. A significant correlation was found between mean blood pressure change and K_i change ($r = 0.46$, $P < 0.025$) (Fig. 3) as well as between mean blood pressure change and estimated myocardial perfusion (LVMI × K_i) change ($r = 0.48$, $P < 0.025$) in response to Dipyridamole. Likewise, there was a significant correlation between heart rate response to deep breathing and K_i change ($r = 0.47$, $P < 0.025$).

DISCUSSION

In the present study, K_i was measured at baseline as well as during Dipyridamole-induced vasodilatation in diabetic patients with and without autonomic neuropathy and in healthy control subjects. Baseline K_i was similar in the three groups, whereas Dipyridamole-induced vasodilatation was significantly smaller in the diabetic patients with autonomic neuropathy than in the diabetic patients without neuropathy, who had an increase in myocardial perfusion that was similar to that of the healthy control subjects. Furthermore, mean blood pressure decreased in response to Dipyridamole in the patients with autonomic neuropathy, in contrast to the two other groups, and there was a significant correlation between blood pressure response to Dipyridamole and K_i change as well as between blood pressure response to Dipyridamole and estimated absolute myocardial perfusion. These findings suggest that diabetic patients with autonomic neuropathy did not increase myocardial perfusion during vasodilatation by Dipyridamole. Lack of an adequate increase in the myocardial perfusion reserve index in patients with autonomic neuropathy may be due to defective vasodilatation caused by a defective sympathetic nervous system, a lack of ability to sustain a normal blood pressure during the Dipyridamole infusion, or a combination of both mechanisms. These findings may provide a pathophysiological link between diabetic autonomic neuropathy and increased cardiovascular mortality associated with this condition.

During the past decade, novel noninvasive techniques have provided new information regarding the impact of autonomic neuropathy on cardiac function in diabetic patients (20). Direct imaging of sympathetic innervation of the heart using measurement of [^{11}C]-hydroxyephedrine or [^{123}I]-metaiodobenzylguanidine (MIBG) retention have demonstrated dysfunction of the sympathetic innervation of the heart very early in the course of type 1 diabetes, and some, but not all, of these changes have been found to be reversible with tight metabolic control (21). These abnormalities in sympathetic innervation of the heart have generally been encountered at a stage in which the simultaneously recorded traditional cardiovascular function tests were within normal range, suggesting that these novel techniques may be more sensitive for the diagnosis

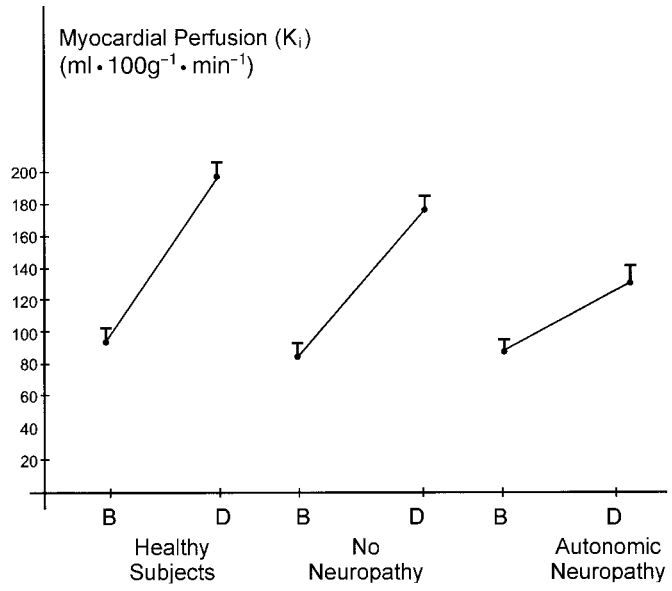

FIG. 1. K_i before (B) and during (D) vasodilatation with Dipyridamole in healthy subjects and in type 1 diabetic patients with and without autonomic neuropathy.

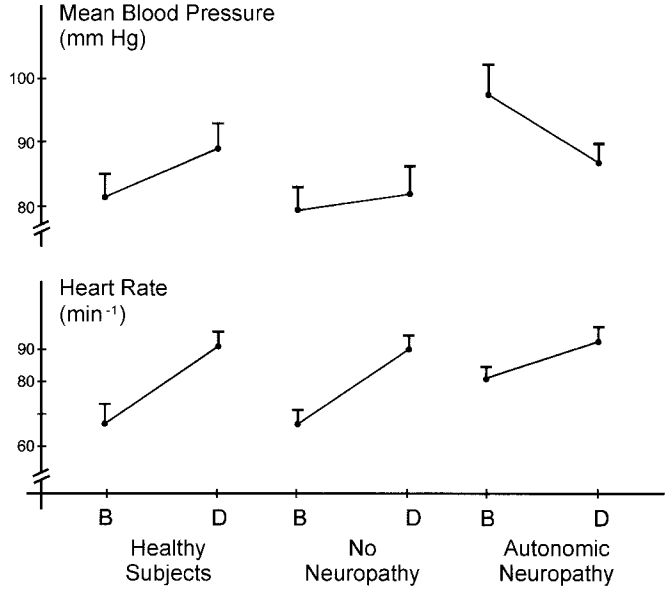

FIG. 2. Heart rate and blood pressure before (B) and during (D) Dipyridamole-induced vasodilatation in healthy subjects and in type 1 diabetic patients with and without autonomic neuropathy.

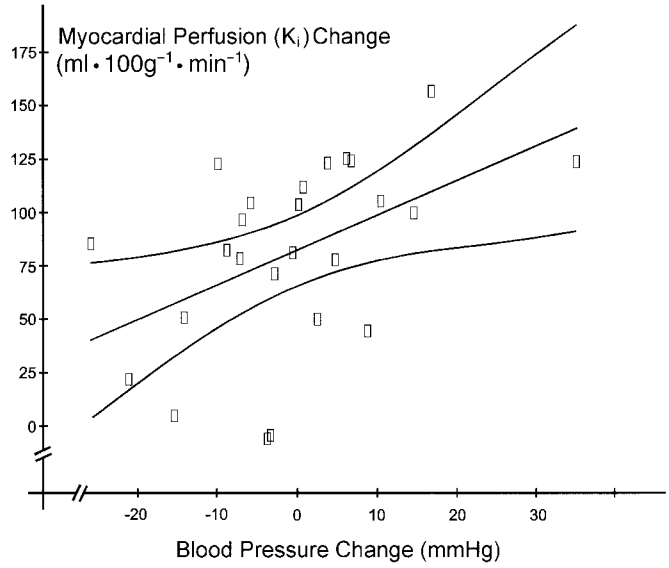

FIG. 3. The relationships between blood pressure response and myocardial perfusion change in healthy subjects and in type 1 diabetic patients with and without autonomic neuropathy.

of autonomic neuropathy (20). However, the natural history of these recently identified abnormalities in cardiac innervation has not been established, nor has the association with the clinical syndromes of diabetic autonomic neuropathy been demonstrated. Changes in methodology for the diagnosis of diabetic autonomic neuropathy await such documentation.

With the advent of positron emission tomography (PET) scan using [^{13}N]ammonia or single-photon emission tomography (SPECT) using [^{201}Th], noninvasive measurements of myocardial perfusion became possible (22–24), and dynamic contrast-enhanced MR myocardial perfusion imaging, as applied in the present study, now adds to the list of methods available for the study of the pathophysiology of diabetic cardiac neuropathy. A significant correlation has been reported between our method of dynamic contrast-enhanced MRI and [^{13}N]ammonia PET measurements of myocardial perfusion in human subjects (25). Using [^{13}N]ammonia, Di Carli et al. (12) found smaller vasodilator response to adenosine in diabetic patients with and without autonomic neuropathy, as defined by [^{11}C]hydroxyephedrine retention, than in healthy subjects. In response to the cold pressor test, the response was even lower in the patients with autonomic neuropathy than in patients without neuropathy, suggesting an association between impaired coronary vasodilatation and cardiac sympathetic dysfunction (12). Likewise, using [^{201}Th] SPECT imaging, the vasodilator response to cold pressor test was impaired in type 2 diabetic patients compared with healthy subjects (26). Meier et al. (21) found, using [^{99}Tc]MIBG-SPECT scintigraphy, that myocardial perfusion did not differ between patients with and without cardiac autonomic neuropathy, the diagnosis being based on conventional cardiovascular tests. The vasodilator response was not studied.

The findings of the present study are in agreement with these studies in the sense that myocardial perfusion during baseline conditions does not differ between diabetic patients with and without autonomic neuropathy. Our findings are, however, to some extent at variance with those of Di Carli et al. (12), who found a similar increase in coronary blood flow in response to adenosine infusion in diabetic patients with and without neuropathy (albeit the coronary blood flow response to the cold pressor test was lower in the patients with autonomic neuropathy). However, the two studies are not comparable regarding classification of autonomic neuropathy, a fact that may explain the different findings.

Changes in sympathetic nervous activity are considered of importance for the coronary blood flow increase in response to vasodilators. This is because increases in coronary blood flow in human cardiac transplant recipients in response to sympathetic stimulation (cold pressor test) correlated with the regional norepinephrine content (as measured by [^{11}C]hydroxyephedrine retention) (27).

Accordingly, the blunted coronary blood flow increase to Dipyridamole might be ascribed to cardiac sympathetic neuropathy. On the other hand, Dipyridamole is a systemic vasodilatator (28), and patients with diabetic autonomic neuropathy had a decrease in mean blood pressure in response to Dipyridamole. A similar hemodynamic response has previously been reported in association with epinephrine infusion, the pathophysiological background most likely being deficient baroreflex response due to autonomic neuropathy (29,30). Therefore, part of the explanation of the defective myocardial perfusion increase could be a Dipyridamole-induced drop in systemic (perfusion) pressure.

Impaired autoregulation of blood flow has been found in several tissues in diabetic patients (31). Intact autoregulation would to some extent upregulate myocardial perfusion in the event of a decrease in blood pressure. Accordingly, part of the decrease in myocardial perfusion increment may be due to defective coronary autoregulation. Coronary artery disease might also contribute to the reduction in myocardial perfusion in the patients with autonomic neuropathy. However, we consider the probability of symptomless coronary artery disease to be low due to lack of clinical signs of cardiac disease, absence of echocardiograhic wall motion abnormalities, and an ejection fraction >0.55, as well as normal ECGs. Definite verification would require coronary arteriography, which was not done for ethical reasons.

In summary, the present study has demonstrated a deficient myocardial perfusion response to a vasodilator (Dipyridamole) in diabetic autonomic neuropathy. The underlying pathogenetic mechanism may be deficient sympathetic-mediated coronary vasodilatation, lack of ability to maintain mean blood pressure during concomitant systemic vasodilatation, or both.

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