Scintigraphic Assessment of Regionalized Defects in Myocardial Sympathetic Innervation and Blood Flow Regulation in Diabetic Patients With Autonomic Neuropathy

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Objectives. **This study sought to evaluate whether regional sympathetic myocardial denervation in diabetes is associated with abnormal myocardial blood flow under rest and adenosine-stimulated conditions.**

Background. **Diabetic autonomic neuropathy (DAN) has been invoked as a cause of unexplained sudden cardiac death, potentially by altering electrical stability or impairing myocardial blood flow, or both. The effects of denervation on cardiac blood flow in diabetes are unknown.**

Methods. **We studied 14 diabetic subjects (7 without DAN, 7 with advanced DAN) and 13 nondiabetic control subjects without known coronary artery disease. Positron emission tomography using carbon-11 hydroxyephedrine was used to characterize left ventricular cardiac sympathetic innervation and nitrogen-13 ammonia to measure myocardial blood flow at rest and after intravenous administration of adenosine (140 μg/kg body weight per min).**

Results. **Persistent sympathetic left ventricular proximal wall innervation was observed, even in advanced neuropathy. Rest myocardial blood flow was higher in the neuropathic subjects (109 ± 29 ml/100 g per min) than in either the nondiabetic (69 ± 8 ml/100 g per min, $p < 0.01$) or the nonneuropathic diabetic subjects (79 ± 23 ml/100 g per min, $p < 0.05$). During adenosine infusion, global left ventricular myocardial blood flow was significantly less in the neuropathic subjects (204 ± 73 ml/100 g per min) than in the nonneuropathic diabetic group (324 ± 135 ml/100 g per min, $p < 0.05$). Coronary flow reserve was also decreased in the neuropathic subjects, who achieved only 46% ($p < 0.01$) and 44% ($p < 0.01$) of the values measured in nondiabetic and nonneuropathic diabetic subjects, respectively. Assessment of the myocardial innervation/blood flow relation during adenosine infusion showed that myocardial blood flow in neuropathic subjects was virtually identical to that in nonneuropathic diabetic subjects in the distal denervated myocardium but was 43% ($p < 0.05$) lower than that in the nonneuropathic diabetic subjects in the proximal innervated segments.**

Conclusions. **DAN is associated with altered myocardial blood flow, with regions of persistent sympathetic innervation exhibiting the greatest deficits of vasodilator reserve. Future studies are required to evaluate the etiology of these abnormalities and to evaluate the contribution of the persistent islands of innervation to sudden cardiac death complicating diabetes.**

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Diabetic autonomic neuropathy (DAN) commonly complicates diabetes (1,2) and is associated with increased mortality rates that range between 16% and 53% over 5 years (3–9). Classically DAN is associated with an overall decrease in myocardial sympathetic innervation (7,8). Functional cardiac abnormalities in diabetic patients without coronary artery disease (CAD) include defects in ventricular function (10–13) and rhythm disturbances (14,15) that correlate with the presence of DAN (12,13). In nondiabetic patients, autonomic imbalance and in particular regional cardiac sympathetic hyperactivity increases the risk of cardiac arrhythmias during myocardial ischemia (16–18). Selective cardiac sympathetic denervation or beta-adrenergic blocking agents reduces the risk of arrhythmias and sudden death in these patients (18). Thus, cardiac sympathetic hyperactivity in combination with regional or generalized myocardial perfusion defects may confer significant added risk of cardiac morbidity or mortality, or both. Whether cardiac sympathetic hyperactivity itself contributes to impaired myocardial blood flow is controversial, because both increased (19) or unchanged flow (20) has been reported after experimental cardiac denervation. In diabetic patients, the increased cardiac mortality (3–9,21–24) paradoxically appears to be further augmented by diminished sympathetic activity in the presence of DAN (3,6–9,21), which may

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Abbreviations and Acronyms

C-11 HED = C-11 hydroxyephedrine
CAD = coronary artery disease
DAN = diabetic autonomic neuropathy
HRV = heart rate variability
PET = positron emission tomography

facilitate malignant cardiac arrhythmogenesis (3–9). Moreover, diabetic patients demonstrate greatly enhanced cardioprotection from beta-blockade (21–23), suggesting that adrenergic hyperactivity contributes to the exaggerated cardiac risk in these subjects.

The myocardial blood flow response to sympathetic activation and consequent norepinephrine release is complex: coronary alpha–adrenoreceptor-mediated vasoconstriction is opposed by alpha$_2$- and beta-adrenoreceptors mediating vasodilation and also by localized release of vasodilator metabolites (25), including adenosine (26). The net effect of sympathetic activation normally is coronary vasodilation (27–29). However, several observations suggest that this balance could be deranged and possibly reversed by diabetes. First, subjects with DAN demonstrate accentuated regional myocardial autonomic imbalance, because left ventricular sympathetic denervation is heterogeneous (12,30). Second, diabetes is associated with impaired endothelium-dependent vascular relaxation (31–34) such that the release of vasodilating agents is impaired, thereby allowing the vasoconstrictive response to adrenergic agonists to predominate (28,29). We hypothesize that in diabetes, preserved regional cardiac sympathetic innervation may result in functional regions of hyperinnervation that would restrict local blood flow and may on sympathetic activation precipitate local paradoxical vasoconstriction and ischemia. These regions of retained innervation may therefore become the focus of electrical and chemical instability and promote cardiac arrhythmias and myocardial dysfunction.

Radiolabeled analogues of norepinephrine, which are retained by the sympathetic nerve terminals of the heart (35,36), permit direct spatial mapping of cardiac sympathetic integrity (30). The radiotracer C-11 hydroxyephedrine (C-11 HED), recently developed as a norepinephrine analogue for positron emission tomography (PET) (30,35,36), undergoes highly specific uptake and retention in the sympathetic nerve terminals (35,36). This permits the quantitative regional characterization of sympathetic neuronal dysfunction and loss (30,35,36). Therefore, in order to evaluate the effects of diabetes-induced heterogeneous myocardial sympathetic innervation on myocardial blood flow, we used C-11 HED and PET to directly characterize left ventricular cardiac sympathetic innervation in diabetic patients with and without DAN and compared this to myocardial blood flow measured using N-13 ammonia (37) at rest and after maximal coronary vasodilation with adenosine (38).

Table 1. Clinical Details of the Subjects

	Nondiabetic Subjects (n = 13)	Nonneuropathic Diabetic Subjects (n = 7)	Subjects With DAN (n = 7)
Male/female (no.)	9/4	5/2	2/5
Age (yr)	39 ± 11	42 ± 6	32 ± 9
Range	26–54	35–50	20–45
Diabetes duration (yr)	—	24 ± 12	21 ± 10
HbA1c	—	8.6 ± 0.9	9.8 ± 2.7
Autonomic symptoms	—	—	7
Nephropathy (>500 mg protein/24 h)	—	—	4
Retinopathy (laser treated)	—	—	4
Lipid profile (mg/dl)			
Total cholesterol	167 ± 40	184 ± 45	183 ± 60
LDL cholesterol	108 ± 32	131 ± 52	142 ± 19
HDL cholesterol	47 ± 11	52 ± 14	50 ± 17
Triglycerides	109 ± 74	82 ± 58	163 ± 67

Data presented are mean value ± SD, unless otherwise indicated. DAN = diabetic autonomic neuropathy; HbAlc = hemoglobin Alc; HDL = high density lipoprotein; LDL = low density lipoprotein.

Methods

Patients. Three groups of patients were studied: insulin-dependent (type 1) diabetic subjects without DAN, insulin-dependent diabetic subjects with advanced DAN, and age-matched nondiabetic control subjects. Clinical details are given in Table 1. All diabetic subjects were recruited from the Michigan Diabetes Research and Training Center Clinical Core. This Core includes a Complications Clinic, in which all subjects have evidence of diabetic neuropathy, retinopathy or nephropathy alone or in combination, and a Continuing Care Clinic in which the patients have no evidence of chronic diabetic complications. Approximately 30 consecutive presentations to the Complications Clinic were screened in order to identify the seven eligible subjects with DAN presented herein. Age- and diabetes duration–matched nonneuropathic diabetic subjects were recruited from ~20 consecutive presentations to the Continuing Care Clinic.

The inclusion criteria for all diabetic subjects comprised a diagnosis of type 1 diabetes; age between 20 and 60 years; the absence or presence of DAN (as defined below); and the absence of any risk factors for other causes for neuropathy (determined by a medical history, family history, history of medications, occupational history, history of exposure to toxins, physical and neurologic examinations and laboratory studies). Because the presence of DAN can mask myocardial ischemia, exercise/stress cardiac studies are standardly performed on these high risk subjects before intensifying their insulin therapy or initiating an exercise program. Thus all the subjects with DAN included in this study had undergone negative cardiac stress testing within the 2-year period preceding this study to confirm the absence of CAD. Exclusion criteria included preexisting cardiovascular disease, including CAD, congestive heart failure, known arrhythmias, docu-

Table 2. Neuropathic Details of the Subjects*

	Nonneuropathic Diabetic Subjects (n = 7)	Subjects With DAN (n = 7)
Rest heart rate (beats/min)	71 ± 12	92 ± 13
HRV (beats/min)	20 ± 4	3 ± 1
Valsalva ratio	1.9 ± 0.3	1.17 ± 0.2
Systolic BP fall (mm Hg)	3 ± 5	30 ± 20
Absent galvanic SP (hand/foot)	0/0	5/5

*Difference between groups for rest heart rate, HRV, Valsalva ratio and systolic BP fall is $p = 0.006$, $p = 0.001$, $p = 0.003$ and $p = 0.006$, respectively, one-way analysis of variance. Data presented are mean value ± SD or are number of patients with absent responses. BP = blood pressure; DAN = diabetic autonomic neuropathy; HRV = heart rate variability; SP = skin potential.

mented ventricular structural abnormalities and valvular disease, peripheral vascular disease and uncontrolled hypertension; a history of primary dyslipidemia requiring therapy; a creatinine clearance <70 ml/min; or a history of previous kidney, pancreas or heart transplantation.

The nonneuropathic diabetic subjects (five men, two women) were selected on the basis of having no evidence of chronic diabetic complications (thereby reducing the risk of occult CAD) and having one or no abnormality on standardized cardiac autonomic testing (39–41) and no symptoms of DAN. Subjects with advanced DAN (two men, five women) had symptoms attributable to autonomic neuropathy and a history of hospital admissions with gastroparesis (and abnormal gastric emptying studies). The symptoms that were reported by all subjects with DAN included nausea, early satiety and periodic episodes of intractable vomiting and persistent constipation with periodic episodes of diarrhea; five subjects reported frequent dizziness on standing; and three suffered from periodic episodes of urinary retention requiring catheterization. All subjects with DAN had two or more abnormalities of autonomic function on standardized testing (below the 5th percentile) (39–41) (Table 2). Originally, eight subjects with DAN were recruited, but one was found to have a discrete myocardial perfusion deficit after adenosine stimulation and was excluded from the study. The diabetic subjects were compared with healthy age-matched nondiabetic subjects (nine men, four women).

Patients taking medications (including caffeine) known to interfere with neuronal uptake of norepinephrine analogues stopped taking their medication for at least 2 weeks before all studies. Written informed consent was obtained from all patients and the study protocol was approved by the institutional review board of the University of Michigan.

Autonomic function testing. Cardiovascular autonomic neuropathy was assessed clinically utilizing a battery of standardized autonomic function tests (39–41) (Table 2). All subjects fasted for at least 8 h before testing and were instructed to delay their morning insulin injection until after testing. Subjects were excluded if they had experienced a hypoglycemic episode within 8 h of testing. Blood glucose values were within the range of 140 to 250 mg/dl during the assessment period. Heart rate variability (HRV) with deep breathing was assessed (39–41) by measuring the maximal and minimal RR intervals and calculating a mean value for the six cycles. A 5-min postural study was performed in which the systolic and diastolic blood pressures were recorded three times at 1-min intervals with the patient supine and the change in pressures was recorded at 1-min intervals for 5 min after standing. The lowest standing systolic pressure was used for calculation of postural change in blood pressure. The Valsalva maneuver (39) was performed and the ratio of the longest RR interval after the maneuver to the shortest RR interval during the maneuver was calculated. This test was repeated twice with a 5-min period of rest between Valsalva maneuvers and the mean value calculated. Abnormalities on these standardized tests were defined as results that were below the 5th percentile for age (39–41). Skin galvanic responses were recorded from the hand and foot (42). These skin responses are a measure of peripheral sympathetic autonomic function because they originate from synchronized activation of sweat glands by efferent sympathetic nerve fibers (42). Stimuli consisted of auditory startle and Valsalva maneuver. Patient responses were recorded as present or absent.

PET studies and image construction. Cardiac PET imaging was performed in a whole body PET scanner (model CTI 931 and Siemens/ECAT 931) with 20 mCi C-11 HED and 20 mCi N-13 ammonia over 60 min (30,37). This scanner has eight circular detector rings that allow for the simultaneous acquisition of 15 contiguous transaxial images (oriented perpendicular to the sagittal and coronal planes of the body) with a slice thickness of 6.75 mm. After placement of a 22-gauge intravenous cannula in the antecubital vein and positioning the tomograph with the aid of a scout image, a 15-min transmission study using a retractable germanium-68 ring source was performed to subsequently correct emission data for tissue attenuation. Tracer was injected intravenously and data acquired dynamically in frame mode to determine tracer activity in both blood and myocardium (30). After waiting 1 h for the C-11 HED decay after the end of data acquisition, myocardial perfusion at rest was evaluated using N-13 ammonia. Emission data were corrected for attenuation and reconstructed using filtered backprojection utilizing a Hanning filter with a cutoff frequency of 0.3 cycles/pixel. A SUN workstation (SUN Microsystems) was used to realign the images perpendicular to the long axis of the left ventricle, yielding eight short-axis views (slice thickness 0.8 cm) of myocardial tracer distribution extending from the apex to the base of the left ventricle.

Homogeneity of C-11 HED retention. The homogeneity of left ventricular C-11 HED retention was assessed as previously reported (30,36). Dynamic scan acquisition was initiated simultaneously with the injection of C-11 HED and the 60-min imaging protocol comprised 15 images with varying frame duration (6×30 s, 2×60 s, 2×150 s, 2×300 s, 2×600 s, $1 \times 1{,}200$ s). Circumferential count-profile analysis was performed on each of the eight short-axis images. Each short-axis slice was divided into 36 angular regions of interest ("sectors")

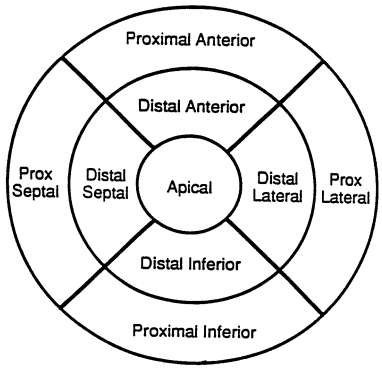

Figure 1. Representation of the left ventricle as a polar map. Individual profiles were generated for each image, and the map was divided into nine regions of relative tracer activity. Prox = proximal.

and the myocardial concentration of C-11 HED in each sector, as reflected by the mean PET counts in the sector, was determined. Regional variation of myocardial retention of C-11 HED was assessed by dividing the mean PET counts in each of the 288 sectors (8 images × 36 sectors/image) by the value found in the sector containing the maximal mean PET counts. These normalized C-11 HED retention data were then displayed as polar coordinate maps of relative tracer activity (C-11/N-13) from the short-axis blood flow and the 30 to 40 min postinjection C-11 HED images. The map was divided into nine regions as shown schematically in Figure 1. In this map, the left ventricular myocardium is depicted with the apex at the center, the distal left ventricular segments (anterior, septal, inferior and lateral) as the inner ring and the corresponding proximal segments as the outer ring. Apical values were obtained by averaging together all sectors in the two most apical short-axis slices. Distal values for the other segments were obtained by averaging together the appropriate sectors in the three planes adjacent to the two apical planes. Similarly, the corresponding proximal values were obtained using the final three short-axis slices toward the base of the heart.

Evaluation of coronary flow reserve. PET evaluation of myocardial blood flow detects perfusion abnormalities with high diagnostic accuracy (43,44). The time course of tracer distribution in myocardium and blood was defined by dynamic image acquisition using a previously described method (38,43). After the transmission scan, 20 mCi N-13 ammonia was administered into a peripheral arm vein over 30 s. Dynamic scan acquisition for this study was initiated with varying frame duration (12 × 10 s, 6 × 30 s, 2 × 300 s). After the baseline N-13 ammonia study, 50 min was allowed for decay of the N-13 ammonia to <3% of its initial activity. Intravenous adenosine was infused into a peripheral vein over 6 min at a dose of 140 μg/kg body weight per min to achieve maximal coronary vasodilation (38,45). At 3 min, 20 mCi N-13 ammonia was injected over 30 s and imaged for 3 to 18 min using the same imaging sequence as the at rest study. Blood pressure and heart rate were measured at baseline, every minute during adenosine infusion, and then at 10 and 18 min. Pressure-rate product was calculated as heart rate times systolic blood pressure divided by 100.

Twelve myocardial regions per plane were defined in the eight planes in the last time frame of the dynamic study sequence. After correction for subject motion, the dynamic image set was sampled and 96 (8 planes × 12 regions) time-activity curves were stored for further analysis. Arterial input function was determined from circular regions at the two most basal planes corresponding to the large blood pool at the center of the largest left ventricle diameter of the resliced images. A previously validated tracer kinetic model for N-13 ammonia (38) was used to calculate myocardial blood flow in ml/g per min for the nine regions shown in Figure 1. This three compartment model represents vascular and extravascular N-13 ammonia as well as metabolically trapped N-13, which comprises glutamine (38). In this model, the delivery and extraction of N-13 ammonia (which is >90% [38]) is used as an estimate of myocardial blood flow, and the flow values generated are in close agreement with those obtained by invasive techniques (38).

Statistical analysis. Statistical analysis was performed using Super ANOVA (Abacus Concepts Inc.). The equality of means of the experimental groups was tested by a one-way analysis of variance, and if significant, the differences were assessed by the Student-Newman-Keuls multiple range test. If the variances for the variables were found to differ significantly, a logarithmic transformation was performed that corrected the unequal variances. All analyses were then performed on the transformed data. Significance was defined at the 0.05 level.

Results

Diabetic subjects with DAN had a higher heart rate at rest, lower HRV and Valsalva ratios, a more pronounced fall in systolic blood pressure on standing and a high frequency of absent galvanic skin responses than the nonneuropathic diabetic subjects (Table 2).

Homogeneity of C-11 HED retention in the normal left ventricle. Studies performed in the normal subjects were processed and averaged together to determine the homogeneity of C-11 HED retention in the healthy left ventricle in order for comparison to be made with the homogeneity of tracer retention in the diabetic subject groups. Relative C-11 HED retention in normal subjects ranged from 69 ± 7% (mean ± SD) of the maximal sector activity value in proximal inferior segments to 82 ± 6% in proximal lateral segments. These regional differences in relative C-11 HED retention did not achieve statistical significance and were consistent with homogeneous uptake of C-11 HED within the normal left ventricle.

Regional C-11 HED retention abnormalities. The heterogeneity of regional left ventricular C-11 HED retention in each diabetic patient was compared with this normal homogeneous distribution by calculating a z-score, $z_i = (q_i - \mu_i)/\sigma_i$, where q_i is the relative C-11 HED retention value in the *i*th sector value of the diabetic polar map, and μ_i and σ_i are the mean and

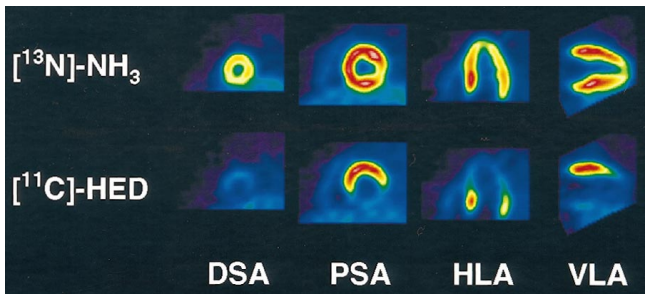

Figure 2. PET images of the left ventricle from a 21-year old woman with DAN who after 13 years of diabetes has symptoms from autonomic neuropathy (gastroparesis and postural hypotension) and abnormalities in all her autonomic function tests. Blood flow images appear normal **(top panels)**, but abnormalities of C-11 HED retention are extensive, with only the proximal cardiac segments being preserved **(bottom panels).** DSA = distal short axis; HLA = horizontal long axis; PSA = proximal short axis; VLA = vertical long axis.

standard deviations of the relative C-11 HED retention in the *i*th sector of the control subjects polar map. In the diabetic subjects, sectors that had a z-score >2.0 (i.e., the relative C-11 HED retention in the patient's sector was less than the corresponding sectors mean relative C-11 HED retention in the normal subjects by >2 SD) were defined as abnormal. Thus, the calculated z-scores represent a validated (30) measure of each subject's myocardial tracer retention heterogeneity, with an increase of heterogeneity being consistent with distal left ventricular denervation (30). The "extent" of the heterogeneity was expressed as the proportion of sectors in the polar map that were abnormal (i.e., $z_i > 2.0$).

Left ventricular sympathetic denervation was significantly more severe in the subjects with DAN than in the diabetic subjects without DAN. The C-11 HED retention abnormalities involved 55 ± 10% of the left ventricle in subjects with DAN compared with 3 ± 1% of the left ventricle in diabetic subjects without DAN ($p = 0.001$). Although four of seven of the diabetic subjects without DAN were found to have small defects in regional C-11 HED retention, none of the deficits involved >5% of the left ventricle. Among the seven subjects with DAN, the left ventricular C-11 HED deficits involved ≤79% of the ventricle. In all subjects with DAN, C-11 HED defects were confined to the distal inferior wall of the left ventricle and progressed proximally with increasing severity of DAN to involve the distal and proximal anterolateral and inferior walls. C-11 HED tracer retention was preserved however in the proximal segments of the anterior and septal left ventricular walls, even in the subjects with the most extensive deficits (Fig. 2).

Assessment for occult regional myocardial perfusion defects. The presence of regional discrete perfusion defects consistent with occult CAD in the study subjects was initially evaluated by visual and semiquantitative analysis of myocardial blood flow at rest and during adenosine-induced vasodilation. Visual inspection of standardized color-coded blood flow images taken from the distal and proximal short axis and the vertical and horizontal long axis confirmed homogeneous N-13 ammonia retention (Fig. 2).

Systemic hemodynamic responses to adenosine infusion in different subject groups. The changes in heart rate, systolic and diastolic blood pressures and pressure-rate product at rest and in response to adenosine infusion are shown in Table 3. At rest the heart rate was higher in the patients with DAN than in either the nondiabetic subjects or the diabetic subjects without DAN. Systolic and diastolic blood pressures were also higher in the subjects with DAN, but this difference did not achieve statistical significance ($p = 0.08$ for systolic blood pressure in nondiabetic vs. subjects with DAN). No differences were observed in the hemodynamic response to adenosine infusion among the subject groups. The rest pressure-rate products were similar in the nondiabetic subjects and diabetic subjects without DAN, but significantly increased in those with DAN. No differences were observed in the pressure-rate product during adenosine infusion. However, the percent change in pressure-rate product was lower in the subjects with DAN than in the diabetic subjects without DAN or nondiabetic control subjects.

Myocardial blood flow at rest and during adenosine stimulation. Myocardial blood flow at rest differed between groups ($p = 0.0003$) (Fig. 3). It was 58% higher in the subjects with DAN (109 ± 29 ml/100 g per min) than in the nondiabetic subjects (69 ± 3 ml/100 g per min, $p < 0.01$) and 38% higher than in the diabetic subjects without DAN (79 ± 9 ml/100 g per min, $p < 0.05$) (Fig. 3A). Rest regional blood flow was higher in all myocardial segments in the subjects with DAN than in

Table 3. Change in Heart Rate and Systolic Blood Pressure Before and After Adenosine Administration*

	Rest	During Adenosine Infusion	% Change in PRP
Nondiabetic subjects			
HR (beats/min)	69 ± 12	89 ± 17	
SBP (mm Hg)	118 ± 18	114 ± 17	
DBP (mm Hg)	76 ± 7	70 ± 11	
PRP	82 ± 15	100 ± 21	24 ± 23
Nonneuropathic diabetic subjects			
HR (beats/min)	71 ± 11	79 ± 11	
SBP (mm Hg)	130 ± 21	129 ± 21	
DBP (mm Hg)	76 ± 18	73 ± 13	
PRP	94 ± 22	105 ± 25	14 ± 10
Subjects with DAN			
HR (beats/min)	96 ± 11†	94 ± 14	
SBP (mm Hg)	142 ± 25	138 ± 30	
DBP (mm Hg)	88 ± 11	78 ± 14	
PRP	128 ± 28†	129 ± 26	1 ± 10‡

*Results are expressed as the mean value ± SD. HR, PRP and % change in PRP differed between groups ($p = 0.0001$, $p = 0.0002$, $p = 0.02$, respectively, one-way analysis of variance). †$p < 0.01$ versus nondiabetic and nonneuropathic diabetic subjects. ‡$p < 0.05$ versus nondiabetic and nonneuropathic diabetic subjects. DAN = diabetic autonomic neuropathy; DBP = diastolic blood pressure; HR = heart rate; PRP = pressure-rate product (HR × SBP/100); SBP = systolic blood pressure.

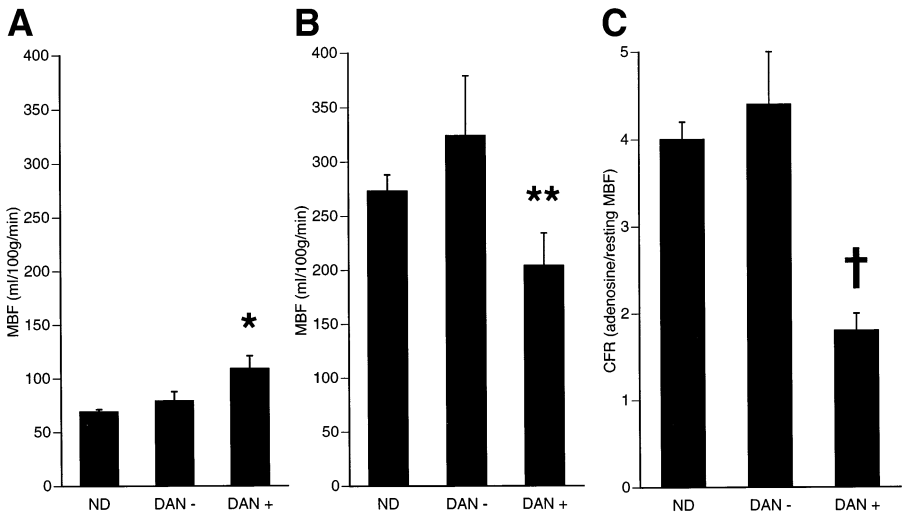

Figure 3. Comparison of myocardial blood flow (MBF) to the whole left ventricle under rest conditions **(A),** during adenosine stimulation **(B)** and the coronary flow reserve (CFR) **(C)** in nondiabetic control subjects (ND), diabetic subjects without DAN (DAN−) and diabetic subjects with DAN (DAN+). Data are shown as mean value ± SEM. *$p < 0.05$ versus other groups. **$p < 0.05$ versus ND. †$p \leq 0.01$ versus other groups.

the other subject groups, with no significant regional differences in flow emerging within the DAN subject group irrespective of the extent of the C-11 HED deficits (Fig. 4). Rest blood flow was similar in nondiabetic subjects and diabetic subjects without DAN. Adenosine-stimulated myocardial blood flow differed between groups ($p = 0.04$). During adenosine infusion, left ventricular myocardial blood flow was significantly less in the subjects with DAN (204 ± 73 ml/100 g per min) than in the diabetic group without DAN (324 ± 135 ml/100 g per min, $p < 0.05$) (Fig. 3B). Although adenosine-stimulated myocardial blood flow in the subjects with DAN was also 25% less than in the nondiabetic subjects (273 ± 26 ml/100 g per min) (Fig. 3B), this difference did not achieve statistical significance ($p = 0.1$). Finally, coronary flow reserve was also found to differ between groups ($p = 0.0001$). In the subjects with DAN, the combination of higher blood flow at rest and a reduction in adenosine-stimulated flow resulted in a significant impairment in coronary flow reserve of only 44% ($p < 0.01$) and 46% ($p < 0.01$) versus nonneuropathic diabetic and nondiabetic control subjects, respectively (Fig. 3C).

Effect of DAN on regional myocardial blood flow during adenosine simulation. In order to explore the relation between heterogeneous myocardial sympathetic innervation and myocardial vascular responsiveness during maximal vasodilator stimulation, rest and adenosine-stimulated regional myocardial blood flow was assessed in the nonneuropathic diabetic subjects and the subjects with DAN in the distal inferior myocardial segments where DAN produced near total denervation (97 ± 2% C-11 HED retention deficit [Fig. 4]) and in the proximal anterior myocardial segments, which were relatively unaffected by DAN (5 ± 4% C-11 HED retention deficit). Rest myocardial blood flow in the denervated distal segments was higher in the subjects with DAN (120 ± 33 ml/100 g per min) than in the diabetic subjects without DAN (80 ± 27 ml/100 g per min, $p = 0.04$). During adenosine stimulation this difference disappeared (254 ± 110 ml/100 g per min vs. 304 ± 178 ml/100 g per min in diabetic subjects with and without DAN, respectively, $p > 0.5$) (Fig. 5, left). Conversely in the innervated proximal myocardial segments, myocardial blood flow at rest was not significantly different between the

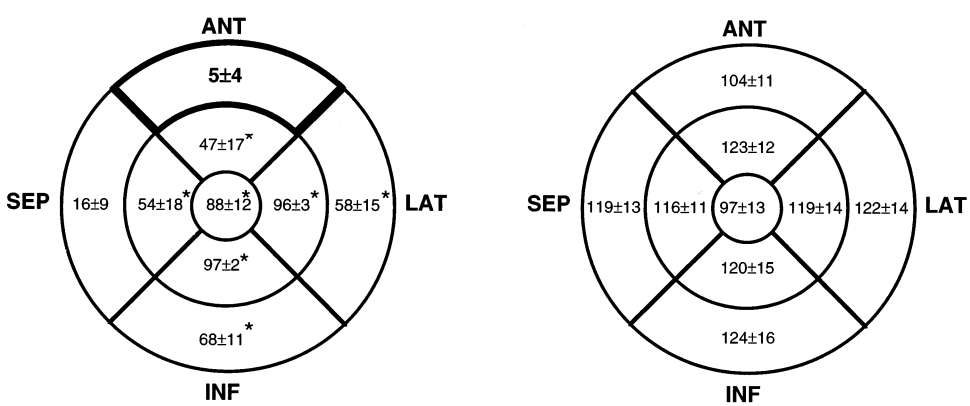

Figure 4. Polar maps of the extent of abnormal C-11 HED retention (shown on the **left**) and rest myocardial blood flow (shown on the **right**) in the nine left ventricular myocardial segments (see Fig. 1) of the diabetic subjects with DAN. Despite a marked heterogeneity of the extent of the regional C-11 HED deficits ($p = 0.0001$, one-way analysis of variance), myocardial blood flow at rest was homogeneous throughout the left ventricle. Data are shown as mean value ± SEM. *$p < 0.01$ versus proximal anterior myocardial segments (in **bold**).

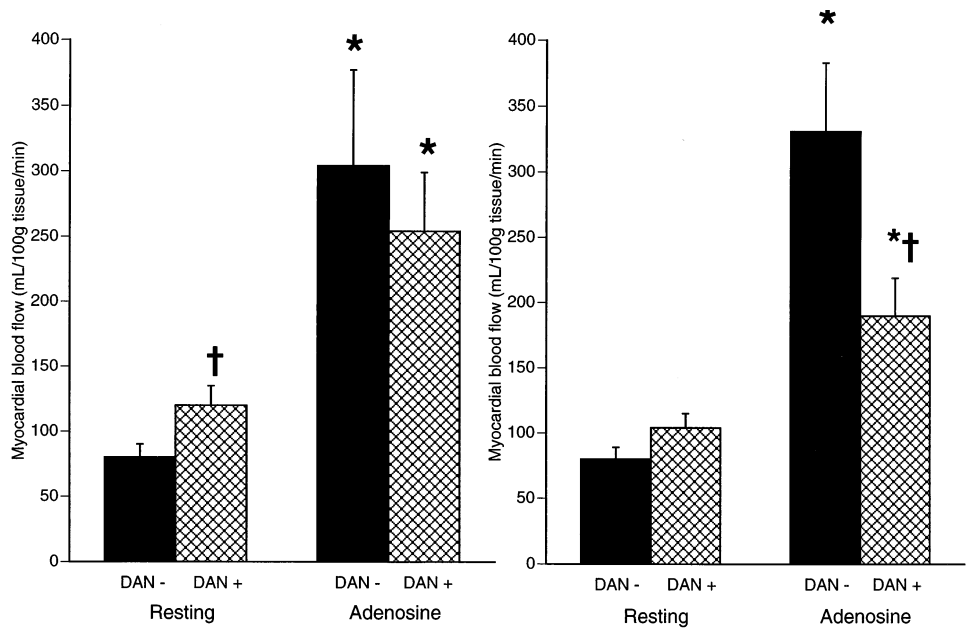

Figure 5. Regional myocardial blood flow under rest conditions and during adenosine stimulation in distal (**left**) and proximal (**right**) myocardial segments. Diabetic subjects without DAN (DAN−) are shown in the **solid** columns and DAN subjects (DAN+) in the **hatched** columns. Data are shown as mean value ± SEM. †$p < 0.05$ versus DAN−. *$p < 0.05$ versus rest flow.

subjects with DAN (104 ± 25 ml/100 g per min) and those without DAN (80 ± 24 ml/100 g per min, $p = 0.2$) (Fig. 5, right), but adenosine-stimulated myocardial blood flow was significantly lower in the subjects with DAN (190 ± 71 ml/100 g per min vs. 331 ± 137 ml/100 g per min in the nonneuropathic diabetic subjects, $p = 0.04$) (Fig. 5, right). No regional differences were observed between nondiabetic and nonneuropathic diabetic subjects under rest or adenosine-stimulated conditions (data not shown).

Discussion

DAN has been invoked as a cause of unexplained sudden death in diabetes (3–9) and may enhance cardiac mortality in diabetic patients with ischemic heart disease (24). To evaluate whether myocardial sympathetic denervation is associated with abnormal myocardial blood flow, we compared global and regional left ventricular sympathetic innervation and blood flow at rest and after maximal coronary vasodilation with intravenous adenosine in normal control subjects and diabetic subjects with and without DAN. The patients with DAN demonstrated heterogeneous distal cardiac sympathetic denervation with persistent proximal innervation. Globally, increased rest myocardial blood flow, decreased adenosine-stimulated myocardial flow and reduced coronary flow reserve accompanied DAN. Reduced global coronary flow reserve in patients with DAN is consistent with the diminished myocardial perfusion described in diabetic patients with angiographically normal coronary arteries and normal left ventricular systolic function (46) (although the neuropathy status was not reported). During adenosine infusion regional myocardial blood flow in neuropathic diabetic subjects was virtually identical to that of the nonneuropathic diabetic subjects in the distal denervated myocardium but was significantly lower in the proximal innervated segments. It is thus tempting to speculate that the persistence of proximal sympathetic innervation is responsible for the regionally blunted vasodilator response to adenosine.

Elevated global myocardial blood flow at rest was restricted to patients with DAN and cannot be attributed simply to the presence of diabetes. This elevated blood flow at rest could have resulted from the tachycardia at rest (Table 2 [39–41]), which would increase rest myocardial oxygen consumption and coronary artery blood flow (47,48). Alternatively, increased rest global myocardial blood flow in subjects with DAN could reflect reduced vasoconstricting sympathetic tone or an imbalance of vasoactive agents, or both. The rest tachycardia commonly reported in DAN (39–41) is conventionally ascribed to a more profound reduction of parasympathetic versus sympathetic tone (39,40). The direct contribution to this process of the preserved proximal sympathetic innervation reported here is unknown. Indeed, the presence of an augmented (rather than simply preserved) regional sympathetic tone stimulating a tachycardia also cannot be conclusively excluded by these studies. Although rest global blood flow was greatly increased, rest regional blood flow was similar in the innervated proximal and denervated distal myocardium of patients with DAN (Fig. 4). The effects of sympathetic denervation on myocardial blood flow are in general controversial. Experimental sympathetic myocardial denervation in dogs has been reported to increase rest myocardial blood flow (19), decrease collateral vessel resistance (49) and increase collateral blood flow by some (50) but not all (20) investigators. Sympathectomy has also been reported to stimulate myocardial capillary angiogenesis in the denervated regions (51,52) and to increase the number of perfused capillaries (53). The

presence of uniform global rest flow in the regionally denervated myocardium of patients with DAN implies that normal innervation is not essential to the preservation of vascularity in the rest state.

In contrast to rest conditions, adenosine-stimulated peak global left ventricular myocardial blood flow and coronary flow reserve were significantly reduced in DAN, implicating myocardial denervation as a factor contributing to impaired stimulated blood flow. Paradoxically, regional myocardial vascular reserve was maximally attenuated in the sympathetically innervated proximal myocardium. Maximal attenuation of vasodilator responsiveness to adenosine in regionally sympathetically innervated segments most likely reflects one or more of the following: regionally exaggerated sympathetic tone, regional vascular hyperresponsiveness or regional histologic microvascular abnormalities. In dogs (28) and normal humans (29), sympathetic activation or acetylcholine infusion (54) results in coronary vasodilation. In diabetes or atherosclerosis, however, endothelial dysfunction may limit the local release of vasodilator agents such as endothelium-derived nitric oxide (31,32) and eicosanoids (33) or increase the release of endothelin-1 (34), permitting a paradoxical vasoconstrictive response to norepinephrine (28,29). Because adenosine is an endothelium-independent vasodilator (37), this study did not directly examine the effects of DAN on endothelial function. Studies using endothelium-dependent vasodilator agents are required to explore these interrelations. Finally, in addition to regional alterations in myocardial sympathetic innervation in subjects with DAN, contributions from regional or global myocardial parasympathetic denervation (6,7,54) (evident from the impaired HRV responses [Table 2]) or a denervation-associated microangiopathy (55,56) cannot be excluded. Therefore present data cannot definitively explain mechanistically the paradoxical adenosine-stimulated myocardial blood flow findings in DAN, but other factors in addition to heterogeneous myocardial sympathetic innervation are probably involved.

Potential clinical implications. The clinical implications of this pattern of regional cardiac sympathetic dysinnervation and impaired vascular reactivity in DAN must be viewed in the light of the following paradox: sympathetic hyperactivity predisposes to sudden death in nondiabetic patients; the hallmark of advanced DAN that confers increased mortality in diabetic patients is sympathetic denervation (6–8).

Diabetes is associated with a two- to threefold increase in the risk of ischemic heart disease and cardiac death, with the greatest risk observed in non–insulin-dependent (type 2) diabetic subjects (57–59). In diabetes, excess cardiac death may be related to sympathetic hyperactivity or imbalance because these patients experience proportionately greater cardioprotection from beta-blockade (21–23) with mortality after infarction being decreased by ≤63% (22). Regional myocardial autonomic denervation and altered vascular responsiveness in DAN may predispose to malignant arrhythmogenesis and sudden cardiac death. In longitudinal studies, the association of DAN with increased mortality (3–9) is highest (7,8) in studies with symptomatic (6) patients with extensive sympathetic and parasympathetic abnormalities on autonomic reflex testing. Most deaths in these patients result from macro- and microvascular disease, such as ischemic heart disease and renal failure, respectively, although cardiorespiratory arrest secondary to autonomic denervation has also been implicated (39,40). Although all the subjects included in the present study had type 1 diabetes, it seems reasonable to speculate that DAN may result in a similar pattern of altered regional vascular responsiveness in type 2 diabetic subjects because these subjects develop an indistinguishable pattern of heterogeneous left ventricular sympathetic denervation (60–62).

In nondiabetic subjects, high cardiac sympathetic tone may predispose to ventricular fibrillation by decreasing the arrhythmogenic threshold (57). Malignant ventricular arrhythmias are associated with cardiac sympathetic hyperactivity, particularly when accompanied by reduced parasympathetic tone and myocardial ischemia (16–18). The crude relative risk of sudden death is doubled (58,59) in these subjects even after correcting for evidence of cardiac ischemia (62). Our studies have shown that cardiac denervation in diabetes closely resembles this pattern of denervation that is thought to predispose to malignant arrhythmias (18,63,64): protective parasympathetic tone is decreased and sympathetic myocardial innervation is most preserved in regions incapable of vasodilating during adenosine stimulation. Our studies do however suggest that in the rest state, the myocardium is well perfused in subjects with DAN (without CAD) and thus circulatory deficiencies should not exacerbate arrhythmogenesis under rest conditions. During stress however, relative regional ischemia in sympathetically innervated regions with diminished parasympathetic protection may be highly arrhythmogenic.

Conclusions. These studies have demonstrated that DAN is associated with altered myocardial blood flow, with regions of persistent sympathetic innervation exhibiting the greatest deficits of vasodilator reserve. Future studies are required to evaluate the etiology of these abnormalities and to evaluate the contribution of the persisting islands of innervation in sudden cardiac death complicating diabetes.

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