Local Sympathetic Denervation in Painful Diabetic Neuropathy

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This study assessed whether painful diabetic neuropathy is associated with abnormal sympathetic nervous function in the affected limbs. Nine patients with diabetes (four men, five women; age 61 ± 7 years) and painful peripheral neuropathy of the feet, but without evidence of generalized autonomic neuropathy, underwent intravenous infusion of tritiated norepinephrine (NE) and sampling of arterial and venous blood in both feet and in one arm to quantify the rate of entry of NE into the local venous plasma (NE spillover). In the same patients, positron emission tomography (PET) scanning after intravenous injection of the sympathoneural imaging agent 6-[^{18}F]fluorodopamine was used to visualize sympathetic innervation and after intravenous [^{13}N]ammonia to visualize local perfusion. The results were compared with those in the feet of normal volunteers and in an unaffected foot of patients with unilateral complex regional pain syndrome (CRPS). In addition, neurochemical results obtained in painful diabetic neuropathy were compared with those obtained in diabetic control patients with painless neuropathy and diabetic control patients without neuropathy. Local arteriovenous difference in plasma NE levels (ΔNE_{AV}) and NE spillover in the arms did not differ across the groups. However, ΔNE_{AV} in the feet was significantly less in the group with painful diabetic neuropathy than in the control groups. Also NE spillover in the feet tended to be lower in painful neuropathy. ΔNE_{AV} of diabetic control patients without neuropathy ($n = 6$) resembled values in the control groups without diabetes, whereas patients with painless diabetic neuropathy ($n = 6$) had evidence suggesting partial loss of sympathetic innervation. PET scanning revealed decreased flow-corrected 6-[^{18}F]fluorodopamine–derived radioactivity in patients with painful diabetic neuropathy, compared with values in normal volunteers and patients with CRPS. The results provide neurochemical and neuroimaging evidence for regionally selective sympathetic denervation in the painful feet of patients with diabetic neuropathy.
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BP, blood pressure; CRPS, complex regional pain syndrome; DHPG, dihydroxyphenolglycol; DOPA, dihydroxyphenylalanine; $\Delta DOPA_{AV}$, l-dihydroxyphenylalanine; ^{18}F:^{13}N ratio, 6-[^{18}F]fluorodopamine-derived radioactivity divided by [^{13}N]-ammonia-derived radioactivity; FBF, forearm blood flow; LBF, leg blood flow; NE, norepinephrine; NE spillover, rate of entry of NE into the local venous plasma; ΔNE_{AV}, arteriovenous difference in plasma NE levels; NIH, National Institutes of Health; PET, positron emission tomography; RSD, reflex sympathetic dystrophy.

Peripheral somatosensory polyneuropathy constitutes a classic long-term complication of both type 1 and type 2 diabetes. The symptoms vary widely and can include pain that ranges from mild to intractable and that can be resistant to treatment (1). Physical findings include decreased or absent ankle deep-tendon reflexes; decreased or absent sensation to touch, vibration, or temperature; and local tenderness. These signs usually are far more pronounced in the feet; the upper extremities are rarely affected.

The cause of the pain is not well understood. Overexcitability or aberrant firing of neuronal sprouts in regenerating neurons is thought to play a role (2), although the occurrence of nerve fiber degeneration and regeneration in itself is unlikely to be sufficient to account fully for diabetic neuropathic pain (3). Drugs that affect sympathetic nervous system function by blocking neuronal uptake of catecholamines (e.g., amitryptiline [4], desipramine [5]) or stimulating α_2-receptors (e.g., clonidine [6]) can decrease the pain, suggesting pathophysiological involvement of the sympathetic nervous system. The findings that the plasma NE level is higher in painful than in painless diabetic neuropathy (7) and that α_1-adrenergic antagonists may be effective in some patients with neuropathic pain (8) lend further support to this idea. The quality of the pain resembles that in patients suffering from complex regional pain syndrome (CRPS), formerly called reflex sympathetic dystrophy (RSD) (9,10).

Findings of increased blood flow in the feet, increased skin blood flow, and increased venous oxygenation (11) have led to the conclusion that at the level of the microcirculation arteriovenous shunting occurs in the feet of patients with diabetic neuropathy (12), regardless of the occurrence of pain. Because precapillary arterioles are under neurogenic control, a pathological increase in arteriovenous shunt flow could indicate "auto-sympathectomy" (12). This hypothesis has not undergone direct testing. Most authors regard generalized sympathetic autonomic neuropathy as a late-occurring phenomenon (13); however, whether localized sympathetic neuropathy attends painful diabetic neuropathy of the feet has been unknown.

To investigate the status of local sympathetic innervation in patients with painful diabetic neuropathy, we used several neurochemical measurements and positron emission tomography (PET) scanning in the same subjects. The neurochemical measurements included regional norepinephrine (NE) spillover, based on intravenous infusion of tracer-labeled NE (14); arteriovenous differences in plasma dihydroxyphenolglycol (DHPG) to indicate local

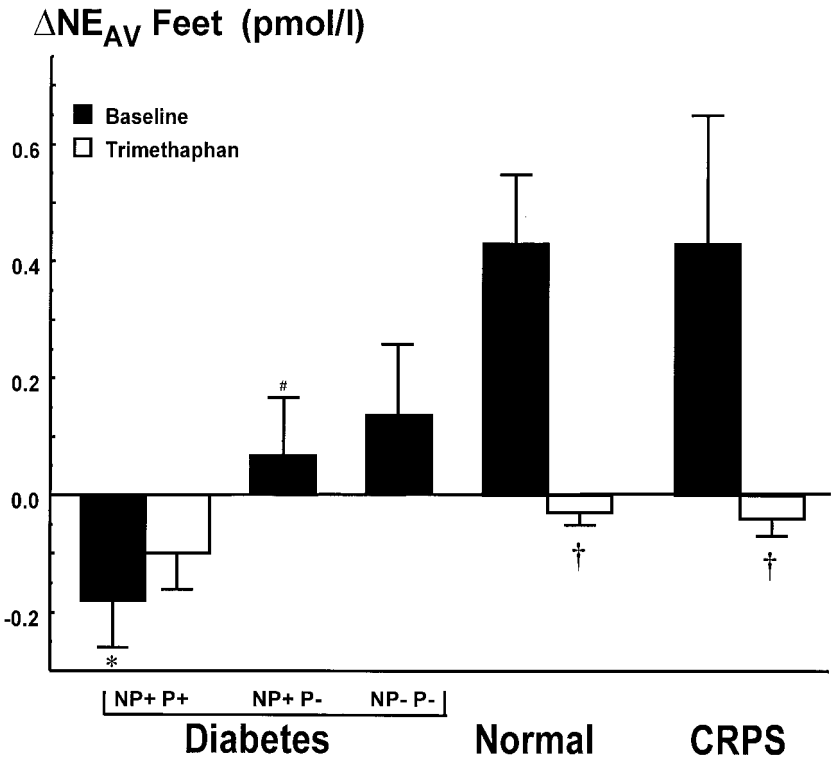

FIG. 1. ΔNE_{AV} under baseline conditions (■) and after ganglion blockade with trimethaphan (□). *Significantly less than normal or CRPS. #Significantly less than CRPS. †Significantly less than baseline.

NE turnover in sympathetic nerves (15), and arteriovenous differences in plasma dihydroxyphenylalanine (DOPA) levels to indicate local NE synthesis (16). PET scanning was done after intravenous [^{13}N]ammonia administration to visualize local perfusion (17) and after intravenous 6-[^{18}F]fluorodopamine administration to visualize local sympathetic innervation (18). Validity of the latter technique was tested in normal volunteers by PET scanning without and with treatment using oral desipramine to block uptake of 6-[^{18}F]fluorodopamine by sympathetic nerves (19). The study also assessed effects of acute sympathoinhibition on the pain and neurochemical indexes using intravenous infusion of the ganglion blocker trimethaphan.

To determine whether findings in diabetic patients were related to painful neuropathy specifically, plasma levels of NE and other catechols were measured in arterial and local venous blood samples in a group of patients without neuropathy and a group with diabetes and nonpainful neuropathy.

RESEARCH DESIGN AND METHODS

Subjects. Five groups of subjects were studied. The first group consisted of nine patients with painful diabetic neuropathy who had called the National Institutes of Health (NIH) for information or were recruited by local advertisement. Patients were accepted after referral from their primary physician and telephone screening. In the NIH Clinical Center, evaluation included a medical history and physical examination, heart rate and blood pressure (BP) responses to deep breathing, standing, and the Valsalva maneuver, as well as blood sampling. BP during the screening examination was recorded continuously using a noninvasive finger cuff device (Finapres). Auscultatory BP was measured in duplicate using a sphygmomanometer, with the patient in the supine position (after at least 5 min bed rest) and then after 1 and 2 min standing. Blood was obtained through an indwelling catheter with the patient lying and standing, for assays of levels of catechols (20).

Patients were continued in the study if they met the following inclusion criteria: *1*) diabetes in stable metabolic control; *2*) polyneuropathy, documented by an abnormal standardized neurological examination (absent ankle reflexes and absent or clearly diminished vibration sense), sometimes complemented by EMG; *3*) pain in both feet; *4*) no evidence of peripheral vascular disease (intact pedal arterial pulses and normal brachial/ankle index by Doppler); *5*) no evidence for alternative causes of neuropathy; and *6*) no signs of sympathoneural failure (i.e., no orthostatic hypotension, normal BP responses to the Valsalva maneuver, and normal increments in plasma NE levels during standing).

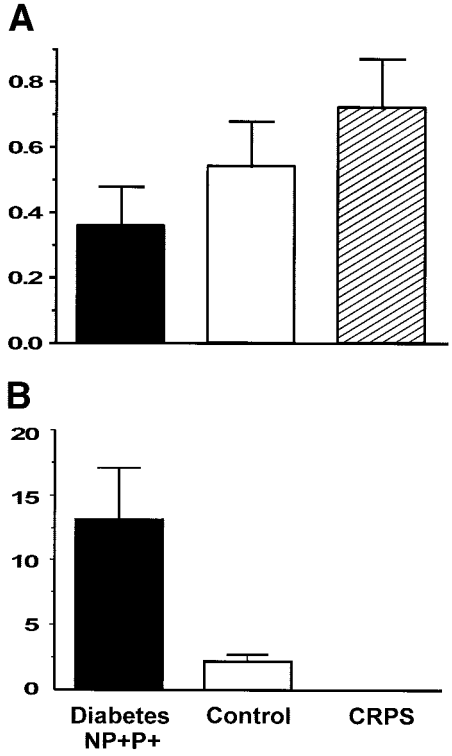

FIG. 2. NE spillover in foot (*A*; pmol · dl^{-1} · min^{-1}) and ratio of arm to foot spillover (*B*). (No ratio for CRPS is given, because the data in arm and foot spillover refer to different individuals.).

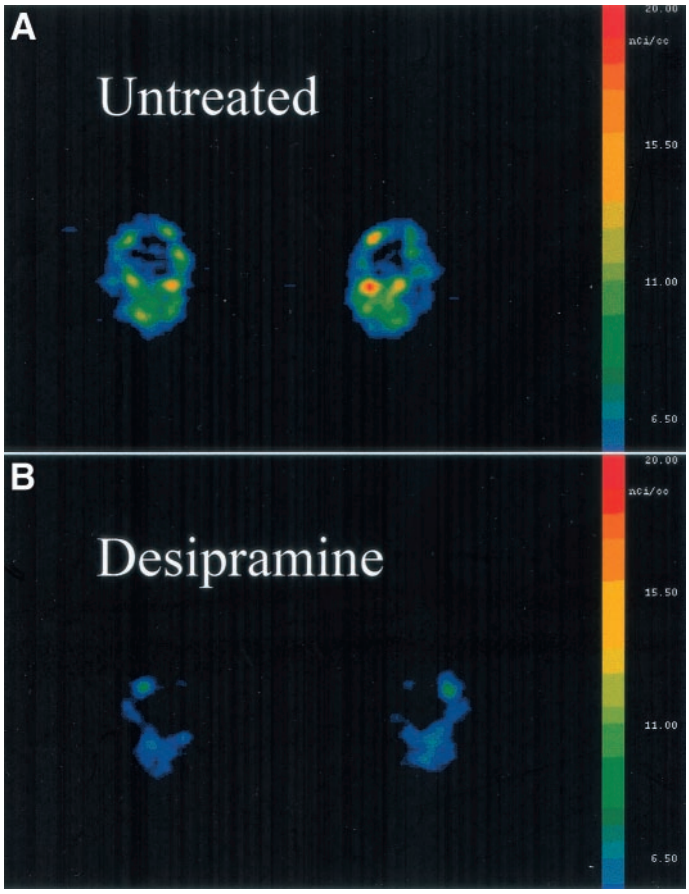

FIG. 3. Representative 6-[^{18}F]fluorodopamine PET scans of the feet of a normal volunteer untreated (*A*) and after treatment with oral desipramine (*B*). The scale for both images is the same.

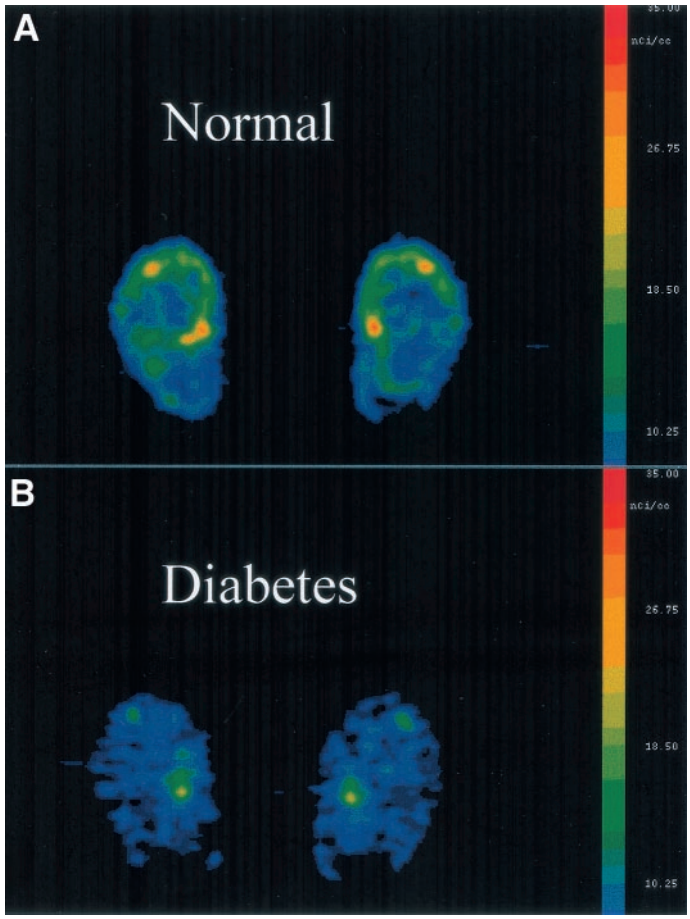

FIG. 4. 6-[^{18}F]Fluorodopamine PET scans of feet of a normal subject (*A*) and of a patient with diabetes (*B*).

The second group consisted of 12 healthy normal volunteers. Of these, eight underwent PET perfusion and sympathoneural scanning of both feet. Of the eight, four underwent PET scanning with and without desipramine in separate PET scanning sessions, in a randomized crossover order. Seven volunteers underwent NE kinetic studies with blood sampled from both feet and from a brachial artery.

The third group consisted of 26 patients with unilateral CRPS (21), in order to assess effects of chronic pain on regional NE spillover. NE spillover was measured in an unaffected leg (n = 15) or an unaffected arm (n = 16). In five patients, both an unaffected leg and an unaffected arm were studied.

The subjects with diabetes or CRPS were studied as inpatients, except for two outpatients. All patients followed a weight-maintaining diet and took their medications as usual. Patients with diabetes discontinued any medications for pain at least five plasma half-lives before the NE kinetic and PET testing sessions. In patients with diabetes, blood glucose was monitored four times daily during the hospital stay. A random plasma glucose was measured in all study subjects. Two patients with CRPS had a previous history of diabetes and were treated with hypoglycemic medication. Their diabetes medication was not discontinued during the studies.

The fourth group consisted of six patients with diabetes and painless diabetic neuropathy, and the fifth group consisted of six patients with diabetes who had no signs or symptoms of neuropathy. These two control groups were matched for age, diabetes duration, sex, and comorbidity with the diabetic group that had painful neuropathy, and they met the same inclusion criteria, except for pain. Patients in these two groups underwent quantitative sensory tests (CASE IV). For the diagnosis of neuropathy, patients were required to have a cold detection threshold or heat-to-pain threshold >90% (22,23). Absence of neuropathy was defined by normal findings during standardized neurological examination and normal values in quantitative sensory testing (i.e., a cold detection threshold or heat-to-pain threshold <75%.

All participants gave written informed consent. The experimental protocol was approved by the Institutional Review Board of the National Institute of Neurological Disorders and Stroke at NIH. The two control groups of patients with diabetes and painless neuropathy or diabetes without neuropathy were studied at the University Medical Center of Nijmegen, the Netherlands, where the experimental protocol was approved by the local ethical committee as well. All assays for plasma levels of tritiated and total catechols were carried out in the same NIH laboratory.

NE kinetics. For assessments of NE kinetics, a brachial arterial catheter was inserted after local anesthesia of the overlying skin. Venous catheters were inserted in a forearm vein, an antecubital vein, and a vein in each foot. Forearm and leg blood flows (FBF and LBF) were measured by strain-gauge venous occlusion plethysmography.

After placement of catheters, [^{3}H]norepinephrine (levo-[2,5,6]-^{3}H-norepinephrine, New England Nuclear, Boston, MA) was infused intravenously at 0.75 μCi/min. After at least 20 min, blood was sampled from the artery, an arm vein, and both foot veins (14,24).

Trimethaphan (2 mg/ml normal saline) was infused intravenously, beginning at a rate of 15 ml/h, with the rate increasing until signs of ganglion blockade were evident. These signs were dry mouth, nasal stuffiness, conjunctival vasodilation, and increased and constant pulse rate. If the diastolic arterial pressure decreased by 10 mmHg or more, then the infusion rate was not increased further, and after taking the flow measurements and blood samples, the infusion was stopped. During ganglion blockade, arterial and venous blood samples were taken again from the arm and feet. Before the infusion and when full ganglion blockade had been attained, patients were asked to score their pain verbally on a scale of 0 (no pain) to 10 (maximum pain ever).

PET scanning. PET scanning was performed on a different day than the NE kinetic study. Each subject was positioned in a GE Advance (General Electric, Milwaukee, WI) scanner, with his or her feet in the gantry. Limb perfusion was assessed by PET scanning for 20 min after a 1-min infusion of 20 mCi of [^{13}N]ammonia. At least 1 h later, 6-[^{18}F]fluorodopamine (specific activity 0.2–1.0 Ci/mmol, dose in most cases 4.0 mCi [25]) was infused intravenously at a constant rate for 3 min, with continuous PET scanning for 30 min.

Four normal volunteers underwent limb PET scanning on separate days, once while untreated and once after treatment with oral desipramine. The sequence of treatments was randomized. Desipramine was administered 30–60 min before the scans, in doses of 25–100 mg (total dose 150 mg in three subjects, 100 mg in one).

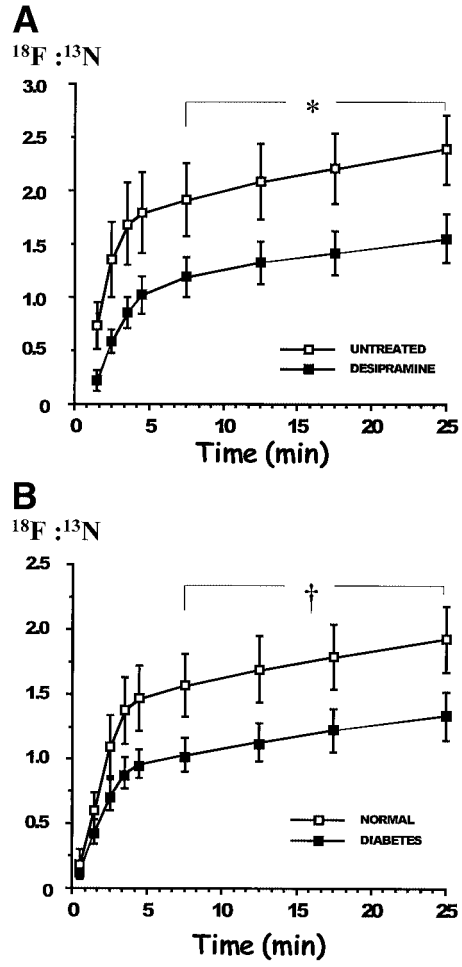

FIG. 5. ^{18}F:^{13}N in normal volunteers before and after desipramine treatment (*A*) and in normal volunteers compared with patients with diabetes (*B*). *Significantly different from untreated across last four time points. †Significantly different from diabetes across last four time points.

Laboratory assays. Arterial and venous plasma was assayed for endogenous and]^{3}H]norepinphrine and other catechols by batch alumina extraction and liquid chromatography with electrochemical detection or fraction collection and liquid scintillation spectrometry (26).

Data analysis and statistics. Limb NE spillover was quantified from the arterial and venous concentrations of total and [^{3}H[norepinephrine and from forearm and leg plasma flows (14,24). In all groups except for the CRPS group, in which data from only the control (unaffected) feet were available, two values for venous and arteriovenous differences of NE, DHPG, and DOPA were obtained, one from the right and one from the left foot. The mean of these two measurements was calculated for each individual and used for further statistical analysis.

PET images of the feet were analyzed as described previously for PET images of the heart (27). Briefly, decay-corrected concentrations (in nanocurie per cubic centimeter) were adjusted for the dose of radioactive drug per kilogram of body weight (in millicurie per kilogram) and expressed in units of $nCi \cdot kg/cc \cdot mCi^{-1}$. Time-averaged (1–30 min for 6-[^{18}F]fluorodopamine, and 5–20 min for [^{13}N]ammonia) images of the same single slices (4.25 mm thick) were obtained at four different levels in the two feet. Radioactivity concentrations in these four transectional slices were averaged in each slice. To provide an index of flow-corrected 6-[^{18}F]fluorodopamine–derived radioactivity, 6-[^{18}F]fluorodopamine–derived radioactivity was divided by [^{13}N]ammonia-derived radioactivity (^{18}F:^{13}N ratio).

Statistical comparisons among the five groups were performed using the Kruskal-Wallis nonparametric test for multiple groups. Post hoc statistical analyses included the Mann-Whitney *U* test for unpaired observations. Paired or unpaired *t* tests were used as appropriate, if the data met the criterion of homogeneity of variance, otherwise Wilcoxon and Mann-Whitney *U* tests were used. For statistical analyses, the SPSS personal computer software package was used, and statistical significance was defined by $P < 0.05$.

RESULTS

Subject characteristics. Of the nine patients with painful diabetic neuropathy (Table 1), two had type 1 diabetes and were treated with insulin, and seven had type 2 diabetes and were treated with diet and oral hypoglycemic agents (four with a sulfonylurea derivative, two with metformin, one with sulfonylurea in combination with troglitazone). Four patients were also treated for hypertension (one with an ACE inhibitor, two with a calcium entry blocker, and one with a thiazide diuretic), and two were treated for hyperlipidemia. None of the patients had either a history or current evidence of orthostatic hypotension.

Plasma NE levels increased in all patients during standing (from 1.60 ± 0.21 to 2.90 ± 0.48 nmol/l after 5 min standing). Patients with diabetes were older and had higher BP than did patients with CRPS and normal volunteers (Table 1). Patients with diabetes weighed more than normal volunteers but not more than CRPS patients.

One of the two CRPS patients, who had CRPS in an upper extremity, had been diagnosed with diabetes and obesity 2 years previously and treated with glyburide. She had no signs of peripheral neuropathy (at the feet). The second CRPS patient had unilateral CRPS in a foot, and had been diagnosed with diabetes and obesity 3 years previously. Her diabetes had been uncontrolled because of nutritional problems, and she had been treated with insulin until recently when she was switched to glyburide in combination with metformin. She had absent ankle reflexes and a slightly diminished vibration sense, but no

TABLE 1
Subject characteristics

	Diabetes				
	Neuropathy, pain	Neuropathy, no pain	No neuropathy, no pain	Control subjects	CRPS
n (M:F)	9 (5:4)	6 (6:0)	6 (3:3)	12 (5:7)	26 (12:14)
Age (years)	60.6 ± 6.8	58.5 ± 7.3	58.7 ± 6.0	35.8 ± 11.7	39.8 ± 7.8
BMI (kg/m^2)	30.8 ± 4.9	28.2 ± 2.0	25.6 ± 4.0	25.9 ± 7.9	28.1 ± 4.8
BP supine (mmHg)	153 ± 24/ 82 ± 7	143 ± 19/ 83 ± 11	147 ± 18/ 78 ± 8	121 ± 17/ 75 ± 8	125 ± 17/ 76 ± 11
Heart rate supine (bpm)	69 ± 10	81 ± 8*	68 ± 8	69 ± 14	74 ± 13
Diabetes duration (years)	13 ± 3	14 ± 6	10 ± 7		
Duration of neuropathy (months)	34 ± 7	54 ± 12	N/A		

Data are means ± SD. *$P < 0.05$, compared with the other diabetic groups. bpm, beats per minute.

TABLE 2
Baseline catecholamine kinetic parameters

	Leg			Arm		
	Diabetes, painful neuropathy	Normal	CRPS	Diabetes, painful neuropathy	Normal	CRPS
NE_v (nmol/l)	1.49 ± 0.17	1.61 ± 0.43	1.86 ± 0.26	1.67 ± 0.23	1.29 ± 0.24	1.57 ± 0.15
ΔNE_{AV} (nmol/l)	−0.17 ± 0.10*	0.43 ± 0.16	0.43 ± 0.22	0.01 ± 0.17	0.09 ± 0.09	0.18 ± 0.08
Fractional extraction	0.36 ± 0.08†	0.56 ± 0.06	0.47 ± 0.08	0.54 ± 0.05	0.72 ± 0.04	0.37 ± 0.05
Blood flow ($pmol \cdot dl^{-1} \cdot min^{-1}$)	1.07 ± 0.13	1.38 ± 0.13	1.80 ± 0.51	3.10 ± 0.64	2.81 ± 0.42	4.30 ± 0.71
Limb NESO ($ml \cdot dl^{-1} \cdot min^{-1}$)	0.34 ± 0.17‡	0.54 ± 0.19	0.72 ± 0.15	1.63 ± 0.54	0.78 ± 0.12	1.58 ± 0.38
$DHPG_v$ (nmol/l)	4.01 ± 0.46	4.37 ± 0.63	5.48 ± 0.66	4.19 ± 0.47	4.63 ± 0.53	4.90 ± 0.32
$\Delta DHPG_{AV}$ (nmol/l)	−0.09 ± 0.08*	0.60 ± 0.35	1.01 ± 0.51	0.09 ± 0.12*	0.40 ± 0.14	0.49 ± 0.11
$DOPA_v$ (nmol/l)	6.38 ± 0.48*	7.60 ± 0.75	8.44 ± 0.77	7.16 ± 0.62	8.56 ± 0.82	8.53 ± 0.55
$\Delta DOPA_{AV}$ (nmol/l)	0.38 ± 0.15*	1.34 ± 0.40	1.45 ± 0.50	1.16 ± 0.19	2.44 ± 0.76	1.61 ± 0.28

Data are means ± SE. *Significantly decreased compared with normal and CRPS. †$P = 0.06$; ‡$P < 0.05$ if each foot considered independently.

pain in the unaffected foot (as measured by the pain syndrome).

Patients with diabetes who had painless neuropathy or did not have neuropathy were matched closely with those who had painful neuropathy, with respect to diabetes duration, duration of neuropathy symptoms/signs (only applicable to the two groups with neuropathy), age, BMI, blood pressure, diabetes treatment (six used oral hypoglycemic agents, six were on insulin), and comedication (two subjects in each group were also treated for hypertension and/or dyslipidemia) (Table 1). The group with diabetic neuropathy but without pain had a significantly higher heart rate than the other two diabetic groups.

Neurochemical assessments

Baseline. Patients with diabetes did not differ from the normal or CRPS groups in arterial NE levels (1.66 ± 0.26, 1.19 ± 0.25, and 1.39 ± 0.10 nmol/l), total body NE spillover (3.39 ± 0.37, 2.88 ± 0.82, and 3.70 ± 0.42 nmol/min), arm venous NE, the arteriovenous increment in NE (ΔNE_{AV}) in the arm, or NE spillover in the arm (Table 2).

In the feet, however, clear group differences emerged. Statistical analysis for several independent samples showed that values for ΔNE_{AV} among the groups of normal volunteers, patients with pain from CRPS, diabetes and painful neuropathy, diabetes and painless neuropathy, and diabetes without neuropathy differed significantly as a function of diagnosis (χ^2 10.3, $P < 0.05$) (Fig. 1 and Table 3). Post hoc analyses showed that the group with diabetes and painful neuropathy had significantly lower values than the normal volunteer and CRPS groups. The group with painless diabetic neuropathy had significantly lower values than the CRPS group but not lower than the normal group, and the group with diabetes who did not have neuropathy did not differ from either the CRPS or normal volunteer group.

For NE spillover in the feet, the group differences were less robust (Table 2 and Fig. 2*A*). When each foot was considered independently, the groups in whom spillover was measured differed significantly (χ^2 7.1, $P = 0.028$), and the patients with painful diabetic neuropathy had significantly less NE spillover than did the control groups. When the values for both feet were averaged in each subject, however, the groups only tended to differ in NE spillover (χ^2 4.5, $P = 0.10$) (Table 2).

In the three groups in whom spillover was measured, mean NE spillover in the arm exceeded that in the foot (Table 2). The ratio of arm to foot NE spillover in the patients with diabetes averaged about six times that in the normal control group (Fig. 2).

While FBF was similar across the five groups, LBF differed significantly (χ^2 12.4, $P = 0.014$), being significantly higher in the group with painless diabetic neuropathy (Table 3).

The five groups also differed significantly in the arteriovenous differences of plasma dihydroxyphenylglycol

TABLE 3
Baseline catecholamine concentrations in patients with diabetes

	Leg			Arm		
	Neuropathy, pain	Neuropathy, no pain	No neuropathy	Neuropathy, pain	Neuropathy, no pain	No neuropathy
n	9	6	6	9	6	6
NE_v (nmol/l)	1.49 ± 0.17	1.15 ± 0.33	1.74 ± 0.33	1.66 ± 0.23	1.14 ± 0.22	1.93 ± 0.33
ΔNE_{AV} (nmol/l)	−0.17 ± 0.10*	0.08 ± 0.12	0.14 ± 0.19	0.01 ± 0.17	0.07 ± 0.05	0.57 ± 0.37
AV-extraction epinephrine (%)	40 ± 7	39 ± 18	55 ± 15	43 ± 8	54 ± 10	29 ± 33
Blood flow ($ml \cdot dl^{-1} \cdot min^{-1}$)	1.07 ± 0.13*	2.42 ± 0.25†	1.91 ± 0.59	3.10 ± 0.64	3.44 ± 0.45	3.04 ± 0.74
$DHPG_v$ (nmol/l)	4.01 ± 0.46	3.17 ± 0.37†	4.30 ± 0.28	4.19 ± 0.47	3.32 ± 0.39	4.80 ± 0.54
$\Delta DHPG_{AV}$ (nmol/l)	−0.09 ± 0.08	−0.91 ± 0.62	−0.13 ± 0.18	0.09 ± 0.12	−0.76 ± 0.77	0.36 ± 0.36
$DOPA_v$ (nmol/l)	6.38 ± 0.48*	7.34 ± 0.94†	8.54 ± 0.53	7.16 ± 0.62	7.55 ± 0.58	8.46 ± 0.37
$\Delta DOPA_{AV}$ (nmol/l)	0.38 ± 0.15*	1.18 ± 0.67	1.29 ± 0.39	1.15 ± 0.19	1.39 ± 0.33	1.20 ± 0.46

Data are means ± SE. *Significantly decreased in patients with neuropathy and pain compared with values in patients without neuropathy and without pain. †Significantly different in patients with neuropathy but not pain compared with values in patients without neuropathy and without pain.

TABLE 4
Catecholamine kinetic parameters during ganglion blockade with trimethaphan

	Leg			Arm		
	Diabetes	Control subjects	CRPS	Diabetes	Control subjects	CRPS
NE_v (nmol/l)	1.00 ± 0.11	0.54 ± 0.11	0.71 ± 0.13	1.02 ± 0.17	0.61 ± 0.15	1.02 ± 0.22
ΔNE_{AV} (nmol/l)	−0.10 ± 0.06	−0.03 ± 0.02	−0.04 ± 0.03	−0.08 ± 0.07	−0.03 ± 0.03	0.20 ± 0.11
Fractional extraction	0.22 ± 0.04	0.17 ± 0.04	0.11 ± 0.08	0.45 ± 0.05	0.59 ± 0.04	0.32 ± 0.07
Blood flow ($ml \cdot dl^{-1} \cdot min^{-1}$)	1.37 ± 0.19	2.31 ± 0.14	2.38 ± 0.82	4.69 ± 1.10	3.99 ± 0.66	5.71 ± 1.95
Limb NESO ($pmol \cdot dl^{-1} \cdot min^{-1}$)	0.10 ± 0.04	0.06 ± 0.03	0.10 ± 0.06	0.99 ± 0.26	0.35 ± 0.08	1.35 ± 0.49
$DHPG_v$ (nmol/l)	3.94 ± 0.44	3.89 ± 0.42	3.96 ± 0.33	4.14 ± 0.48	3.95 ± 0.38	4.54 ± 0.38
$\Delta DHPG_{AV}$ (nmol/l)	−0.05 ± 0.10	0.23 ± 0.12	0.17 ± 0.13	0.15 ± 0.09	0.26 ± 0.10	0.31 ± 0.13
$DOPA_v$ (nmol/l)	6.08 ± 0.47	7.00 ± 0.63	7.00 ± 0.81	6.83 ± 0.46	7.96 ± 0.78	7.80 ± 0.78
$\Delta DOPA_{AV}$ (nmol/l)	0.18 ± 0.07	0.64 ± 0.33	0.54 ± 0.30	0.92 ± 0.20	1.74 ± 0.60	1.19 ± 0.37

Data are means ± SE.

($\Delta DHPG_{AV}$) in the foot (χ^2 10.9, $P = 0.028$), and the arm (χ^2 10.1, $P < 0.05$), because of lower values in the groups with painful and painless diabetic neuropathy (Tables 2 and 3); and the groups tended to differ in the arteriovenous increments in plasma dihydroxyphenylalanine ($\Delta DOPA_{AV}$) in the feet (χ^2 9.3, $P = 0.055$), again because of lower values in the groups with diabetic neuropathy (Table 2); $\Delta DOPA_{AV}$ in the arm was not different across the groups (χ^2 1.9, NS).

The three groups with diabetes did not differ significantly in mean values for $\Delta DHPG_{AV}$, but mean values for both arterial and venous DOPA differed, with significantly lower values in the group with diabetic neuropathy and pain. $\Delta DOPA_{AV}$ showed a borderline significant difference across the three diabetic groups, again with lower values in painful neuropathy (Table 3).

Ganglion blockade. During intravenous infusion of trimethaphan, arterial and venous NE levels and total body and limb NE spillovers decreased significantly as expected in all three groups (Table 4 and data not shown). ΔNE_{AV} decreased significantly during ganglion blockade in normal feet but not in diabetic feet, because of the already low mean value at baseline in the latter group.

During ganglion blockade, values for $\Delta DHPG_{AV}$ and $\Delta DOPA_{AV}$ did not change in patients with diabetes (Table 4). Patients with diabetic neuropathy seemed to be especially sensitive to the hypotensive effect of trimethaphan, since the mean trimethaphan dose to attain ganglion blockade (0.91 ± 0.13 mg/min) was significantly smaller than that in normal volunteers (1.70 ± 0.16 mg/ml) or in patients with CRPS (1.51 ± 0.16 mg/ml, both $P < 0.01$). Even at the lower mean dose, decrements in BP during ganglion blockade were more pronounced in the diabetic subjects than in either control group (Table 5).

Ganglion blockade did not affect the mean pain score of patients with diabetic neuropathy (2.3 ± 1.9 before and 1.8 ± 1.4 during trimethaphan).

PET scanning. In normal volunteers, desipramine reduced 6-[^{18}F]fluorodopamine–derived radioactivity (Fig. 3). [^{13}N]Ammonia-derived radioactivity remained unchanged. As a result, desipramine substantially decreased flow-corrected 6-[^{18}F]fluorodopamine–derived radioactivity. [^{13}N]Ammonia PET scanning showed increased perfusion in the feet of patients with diabetic neuropathy, compared with values in normal volunteers (226 ± 28 vs. 130 ± 20 $nCi \cdot kg/cm^3 \cdot mCi^{-1}$, $P = 0.014$). Flow-corrected 6-[^{18}F]fluorodopamine-derived radioactivity was markedly lower in diabetic feet, with the mean value for ^{18}F:^{13}N about 1.0, not different from the values in desipramine-treated normal volunteers (Figs. 4 and 5).

DISCUSSION

The present results lead to the conclusion that patients with painful diabetic neuropathy have sympathetic denervation in the affected limb.

The findings of a decreased arteriovenous difference in plasma NE levels and decreased NE spillover in the affected foot indicate decreased NE release into the bloodstream from local sympathetic nerve terminals. During ganglion blockade, patients with diabetic neuropathy failed to decrease NE spillover in the affected foot, as one would expect if sympathetically mediated release of NE outflow already was low. Decreased local NE spillover could reflect either decreased NE release from sympathetic nerves or increased efficiency of NE uptake after its release. The finding of, if anything, decreased fractional extraction of circulating [^{3}H]norepinephrine in the af-

TABLE 5
Hemodynamic responses to ganglion blockade

	Diabetes, painful neuropathy		Control subjects		CRPS	
	Basal	Trimethapan	Basal	Trimethapan	Basal	Trimethapan
Systolic BP (mmHg)	178 ± 7	132 ± 10*	129 ± 8	112 ± 5*	134 ± 5	113 ± 5*
Mean arterial pressure (mmHg)	117 ± 5	92 ± 7*	94 ± 5	86 ± 4*	94 ± 4	83 ± 4*
Diastolic BP (mmHg)	82 ± 2	69 ± 5*	73 ± 4	69 ± 4	71 ± 4	67 ± 4
Heart rate (bpm)	77 ± 5	78 ± 5	67 ± 2	87 ± 4*	70 ± 7	84 ± 6*
FVR (AU)	49 ± 10	28 ± 7*	45 ± 8	26 ± 4*		

Data are means ± SE. Forearm vascular resistance (FVR) is mean arterial pressure divided by foot blood flow, in arbitrary units (AU). Data for foot blood flow unavailable for some CRPS patients. *$P < 0.05$ vs. basal.

fected foot supports the former explanation. The extent of the decreases in arteriovenous increments and NE spillover probably underestimates the extent of decreased local release of NE, because of concurrently decreased extraction of NE.

DHPG is the main neuronal metabolite of NE (15). Under resting conditions, plasma DHPG levels mainly reflect turnover of NE in vesicles in sympathetic nerves (28). The finding of locally decreased release of DHPG in the affected limb, reflected by low values for $\Delta DHPG_{AV}$, indicated a decreased pool size of NE in sympathetic nerves. This pool size would not change during acute ganglion blockade. Thus, low DHPG production was present in the patients with painful diabetic neuropathy, with or without trimethaphan infusion.

Under steady-state conditions, synthesis balances turnover of neurotransmitter stores. The arteriovenous increment in plasma levels of l-dihydroxyphenylalanine ($\Delta DOPA_{AV}$) reflects local synthesis of NE (16) and correlates positively with $\Delta DHPG_{AV}$ (29). The finding of low values for $\Delta DOPA_{AV}$ in the affected limbs of patients with diabetic neuropathy therefore indicated coupled decreases in synthesis and turnover of NE in the affected feet of patients with diabetic neuropathy. The neurochemical pattern of decreased $\Delta DOPA_{AV}$ and decreased $\Delta DHPG_{AV}$ excluded decreased turnover from interference with the vesicular monoamine transporter (30). Decreased NE release in patients with diabetic neuropathy therefore does not arise from a "reserpine-like" depletion of NE stores.

The most straightforward and likely explanation for the neurochemical findings indicating locally decreased release, neuronal uptake, turnover, and synthesis of NE in painful diabetic neuropathy is the loss of sympathetic postganglionic innervation. The results of sympathetic neuroimaging using 6-[^{18}F]fluorodopamine PET scanning confirmed this explanation. Since desipramine treatment markedly reduced 6-[^{18}F]fluorodopamine–derived radioactivity in the feet of normal volunteers, without affecting local perfusion, as indicated by [^{13}N]ammonia PET scanning, local concentrations of 6-[^{18}F]fluorodopamine-derived radioactivity reflect uptake and vesicular storage of 6-[^{18}F]fluorodopamine in sympathetic nerves. Patients with painful diabetic neuropathy had decreased perfusion-adjusted 6-[^{18}F]fluorodopamine–derived radioactivity, to values similar to those of desipramine-treated normal volunteers. The neurochemical and PET results therefore strongly suggest that, even in the absence of symptoms, signs, or neurochemical evidence of generalized autonomic neuropathy, patients with painful diabetic neuropathy have sympathetic denervation in the affected limb.

Literature on this topic provides indirect support for this view. Peripheral sympathetic denervation may explain the microvascular changes found in patients with diabetic neuropathy (11,12). Abnormalities in the microcirculation have been demonstrated in patients who had no evidence of autonomic neuropathy detected by standard cardiovascular autonomic function tests (31). It should be realized that investigation of peripheral sympathetic nervous function by laser-Doppler flowmetry and transcutaneous oxygen tension measurement is hampered by large intra- and interindividual variability and by several systemic and local factors (32). The findings in this study provide more direct evidence for a decrease in regional sympathetic nervous tone.

The three study groups were not well matched for weight, age, or BP, and each of these factors has been reported to be related to local or systemic release of NE. NE spillover in the total body and in the limbs increases as a function of normal aging (33,34). The mismatch in the groups mean age would be expected if anything to obscure the decreased NE spillover in the foot of diabetic patients. Values for $\Delta DHPG_{AV}$ and $\Delta DOPA_{AV}$ do not change as a function of aging (35). Directly recorded skeletal muscle sympathetic activity and plasma NE levels increase with increasing body mass (36); however, $\Delta DHPG_{AV}$ and $\Delta DOPA_{AV}$ do not (37). Whether patients with chronic hypertension have increased sympathetic outflows has aroused persistent controversy (38). The best evidence for abnormal sympathoneural function comes from studies of relatively young patients with borderline hypertension (39). An artifactual effect of mismatching the diabetic and control groups for BP would, if anything, obscure decreased local NE spillover in the affected limb.

None of these potential limitations apply to the high arm to foot ratio of NE spillover in patients with painful diabetic neuropathy. This ratio normally averages ~2 (40); however, in the present study, the ratio in patients with painful diabetic neuropathy averaged ~12, six times that in normal volunteers.

The patients with diabetes in our study were selected based on clinical evidence of painful peripheral neuropathy. Some authors distinguish two forms of painful diabetic neuropathy: acute and chronic (41). Acute pain is thought to be related to changes in metabolic control ("insulin neuritis") and improves with prolonged good glycemic control. The present study included only patients in stable metabolic control. These patients had a combination of pain and decreased pain sensation—sometimes also called the "painful painless" foot (42). The results of the neurochemical analyses in the group of neuropathic patients who did not have pain and the findings in the CRPS patient who had asymptomatic diabetic neuropathy suggest that peripheral sympathetic denervation occurs in these patients as well (43). Because ΔNE_{AV} in the diabetic subjects without neuropathy was not significantly different from that in normal and CRPS subjects, and because the NE spillover in the diabetic CRPS patient without neuropathy was in the normal range as well, there is little support for the possibility that the diabetic state in itself might cause the abnormalities.

The absence of improvement in reported pain during ganglion blockade makes it unlikely that the sympathetic nervous system actually contributes to the pain in painful diabetic neuropathy. Consistent with this view, an old report by Joslin (44) noted that surgical sympathectomy was not beneficial in this setting. In streptozotocin-induced diabetic rats, hyperalgesia does not appear to be related to the sympathetic nervous system (45). The previously reported beneficial effect of tricyclic antidepressant drugs in this condition may therefore arise from blockade of catecholamine reuptake in the central nervous system (46). Sympathetic denervation could enhance pain transmission if sympathetic nerves and nociceptors competed for uptake or binding of neurotrophic factors. Acute

sympathetic peripheral nerve damage increases expression of nerve growth factor in the dorsal root ganglion (47). Whether chronic sympathetic neuropathy alters expression of neurotrophic factors, augmenting pain sensation, is unknown.

In contrast with PET findings in the heart, 6-[^{18}F]fluorodopamine–derived radioactivity in the feet increased during the 30-min period of PET scanning. Since arterial plasma levels of 6-[^{18}F]fluorodopamine fall extremely rapidly after cessation of the infusion (half-time ~1.4 min [25]) (25), the accumulation of 6-[^{18}F]fluorodopamine–dervied radioactivity did not result from continued uptake of the tracer itself. The progressive increases in 6-[^{18}F]fluorodopamine–derived radioactivity were similar in desipramine-treated and in untreated normal volunteers, suggesting probable non-neuronal local uptake of metabolites of 6-[^{18}F]fluorodopamine.

In summary, patients with painful diabetic neuropathy of the feet have neurochemical and PET scanning evidence for local sympathetic denervation. The pain is not sympathetically mediated. The ability to assess the extent of neuropathy quantitatively may facilitate future studies about efficacy of drug treatments for this condition.

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