Heterogeneous Cardiac Sympathetic Denervation and Decreased Myocardial Nerve Growth Factor in Streptozotocin-Induced Diabetic Rats

Implications for Cardiac Sympathetic Dysinnervation Complicating Diabetes

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Heterogeneous myocardial sympathetic denervation complicating diabetes has been invoked as a factor contributing to sudden unexplained cardiac death. In subjects with diabetic autonomic neuropathy (DAN), distal left ventricular (LV) denervation contrasts with preservation of islands of proximal innervation, which exhibit impaired vascular responsiveness. The aims of this study were to determine whether this heterogeneous pattern of myocardial sympathetic denervation occurs in a rat model of diabetes and to explore a potential association with regional fluctuations in myocardial nerve growth factor (NGF) protein. Myocardial sympathetic denervation was characterized scintigraphically using the sympathetic neurotransmitter analog C-11 hydroxyephedrine ([^{11}C]HED) and compared with regional changes in myocardial NGF protein abundance and norepinephrine content after 6 and 9 months in nondiabetic (ND) and streptozotocin-induced diabetic (STZ-D) rats. In ND rats, no difference in [^{11}C]HED retention or norepinephrine content was detected in the proximal versus distal myocardium. After 6 months, compared with ND rats, myocardial [^{11}C]HED retention had declined in the proximal segments of STZ-D rats by only 9% (NS) compared with a 33% decrease in the distal myocardium ($P < 0.05$). Myocardial norepinephrine content was similar in both ND and STZ-D rats. At 6 months, LV myocardial NGF protein content in STZ-D rats decreased by 52% in the proximal myocardial segments ($P < 0.01$ vs. ND rats) and by 82% distally ($P < 0.01$ vs. ND rats, $P < 0.05$ vs. proximal segments). By 9 months, [^{11}C]HED retention had declined in both the proximal and distal myocardial segments of the STZ-D rats by 42% ($P < 0.01$ vs. ND rats), and LV norepinephrine content and NGF protein were decreased in parallel. Therefore, 6 months of STZ-induced diabetes results in heterogeneous cardiac sympathetic denervation in the rat, with maximal denervation occurring distally, and is associated with a proximal-to-distal gradient of LV NGF protein depletion. It is tempting to speculate that regional fluctuations of NGF protein in the diabetic myocardium contribute to heterogeneous cardiac sympathetic denervation complicating diabetes.
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Diabetic autonomic neuropathy (DAN) commonly complicates diabetes and has been invoked as a cause of sudden death in diabetic subjects both with and without myocardial ischemia (1–3). In diabetes, the excess cardiac mortality (3–7) appears to be further augmented by DAN (3,6), particularly in patients with advanced deficits of sympathetic cardiovascular innervation, which may facilitate malignant cardiac arrhythmogenesis (7), although direct evidence is lacking. Radiolabeled analogs of norepinephrine are actively taken up by the sympathetic nerve terminals of the heart and thereby permit direct regional assessment of cardiac sympathetic integrity. Cardiac scanning with the radiotracers [^{123}I]metaiodobenzylguanidine (MIBG) (8–10) and C-11 hydroxyephedrine ([^{11}C]HED) (11–13) has identified myocardial sympathetic denervation in diabetic subjects even with normal cardiovascular reflex testing (10–13). These studies have demonstrated heterogeneous left ventricular (LV) sympathetic denervation with maximal deficits occurring distally but relative sparing of the proximal myocardium. Since the proximal myocardial segments of retained innervation may show paradoxically increased tracer retention (12) consistent with functional hyperinnervation and impaired vasodilatory reserve (13), these regions could potentially become a focus of electrical and chemical instability.

The etiology of cardiac sympathetic dysinnervation in diabetes is unknown but may reflect disruption of the metabolism of the potent neurotrophic factor nerve growth factor (NGF). NGF protein and mRNA are found in target organs of sympathetic innervation, including the heart (14,15). Retrograde transport of NGF from target organs to neuronal cell bodies is required for normal growth, maintenance, and regeneration of the peripheral nervous system (16,17). NGF's actions are largely mediated by binding to its high- and low-affinity recep-

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BSA, bovine serum albumin; DAN, diabetic autonomic neuropathy; [^{11}C]HED, C-11 hydroxyephedrine; LV, left ventricular; MIBG, [^{123}I]metaiodobenzylguanidine; ND, nondiabetic; NGF, nerve growth factor; STZ, streptozotocin; STZ-D, streptozotocin-induced diabetes.

tors (18–20). Deletion of the high-affinity NGF receptor *trk*A in mice results in extensive loss of sympathetic ganglionic neurons (18). In the rat heart, NGF mRNA and protein levels correlate with density of sympathetic innervation (14,15). Hypothetically, dense innervation of the proximal LV and elevated levels of NGF may contribute to the resistance of the proximal myocardium to denervation in diabetes.

Depletion of NGF has recently been implicated in the pathogenesis of sensory and autonomic neuropathy complicating diabetes. In diabetic rodents (21,22), serum NGF levels are reduced and NGF protein levels are decreased after 4 weeks in some sympathetically innervated target organs including the sciatic nerve (22,23). Additionally, decreased expression of NGF receptors in dorsal root ganglia of streptozotocin-induced diabetic (STZ-D) rats (24) supports a role for impaired NGF-mediated neurotrophism in the pathogenesis of experimental diabetic neuropathy. Conversely, in the atria and composite ventricles, NGF protein content increases 4 weeks after the induction of diabetes (22), which may reflect impaired axonal transport or possibly increased regional synthesis (22,25,26). In STZ-D rats, increased myocardial NGF (22) correlates with acute elevations of myocardial norepinephrine (27–29), which subsequently return to nondiabetic (ND) levels. Although in diabetic rats, NGF protein levels eventually decline in the total left ventricle below ND values after 12 weeks of diabetes (22), NGF (like norepinephrine [27]) remains elevated in the densely innervated atria (22). It is unknown whether there are regional changes of NGF protein in the left ventricle of diabetic rats.

The aims of this study were therefore to determine whether the STZ-D rat can be used as a model of human heterogeneous myocardial sympathetic denervation and to explore potential relationships with myocardial NGF. To this end, the time-dependent changes in regional LV sympathetic innervation in the STZ-D rat heart were determined using [^{11}C]HED and compared with regional fluctuations in myocardial NGF protein content.

RESEARCH DESIGN AND METHODS

Animal model. Male Wistar rats (180–200 g) were acclimatized for 1 week before being fasted overnight and rendered diabetic by an intraperitoneal injection of 50 mg/kg STZ (Upjohn, Kalamazoo, MI) in 0.2 ml of 10 mmol/l citrate buffer, pH 5.5. Diabetes was defined as a nonfasting plasma glucose >250 mg/dl in tail vein blood (One Touch II; Lifescan, Milpitas, CA) 48 h after STZ injection. Animals were subsequently maintained for a time period of 6 months (ND, n = 15; STZ-D, n = 18) or 9 months (ND, n = 11; STZ-D, n = 11) in individual air-filtered metabolic cages with ad libitum access to water and fed a standard rat diet (ICN Biomedicals, Cleveland, OH). When necessary, STZ-D rats received small daily doses (0.5–3.0 U) of protamine zinc insulin to maintain blood glucose between 350 and 450 mg/dl. At the end of the experimental period, ND and STZ-D animals were divided into two groups, one for assessment of myocardial [^{11}C]HED retention and the other for measurements of myocardial norepinephrine and NGF protein content. All end-point measurements were made by investigators who were unaware of the identity of the experimental assignment.

Regional retention of [^{11}C]HED. The integrity of sympathetic innervation was evaluated at 6 (ND, n = 5; STZ-D, n = 9) and 9 (ND, n = 5; STZ-D n = 5) months by assessing regional myocardial retention of [^{11}C]HED using a gamma counter (Packard Instruments, Meriden, CN). Neuronal heart imaging was performed using a bolus of 200 µCi [^{11}C]HED injected into the femoral vein under ether anesthesia. After 30 min, the animal was weighed and killed by decapitation, and the heart was rapidly removed, rinsed with saline, and blotted dry. The left ventricle was divided into basal and apical sections, which together with the right ventricle were placed into preweighed tubes and weighed. Radioactivity was measured in each segment and a correction made for decay. The results are expressed as percent kilogram dose per gram, which normalizes for differences in body weight (kg) and provides the concentration of tracer in the tissue.

Myocardial norepinephrine. Myocardial norepinephrine levels were determined at 6 (ND, n = 10; STZ-D, n = 9) and 9 (ND, n = 6; STZ-D n = 6) months as described by Karlsson et al. (30). In brief, tissue samples of 100 mg were homogenized in 0.1 mol/l perchloric acid. After protein removal by centrifugation and the addition of Tris-HCL (pH 8.6), the extracts were shaken for 20 min with 10 mg of previously reactivated acid-washed alumina (Sigma, St. Louis, MO). Dihydroxybenzylamine was used as the internal standard. After allowing the alumina to settle and washing, catecholamines were eluted with 0.1 mol/l perchloric acid. Norepinephrine was isolated from the eluates by reverse-phase high-performance liquid chromatography (HPLC). Norepinephrine and the internal standard were quantitated with an electrochemical detector. Recovery of internal standard was consistently >80%.

NGF protein. Regional myocardial NGF protein content was determined in parallel to norepinephrine levels on the same animal sets given above. Heart extracts were prepared by Polytron tissue disruption at a maximal weight-to-volume ratio of 1:10. A high-salt, low-detergent extraction buffer was used (31). Homogenates were divided, and one was spiked with 500 pg/ml NGF-β (Sigma) and centrifuged at 13,000*g*, 4°C, for 20 min. MaxiSorp 96-well plates were coated with a solution containing 50 mmol/l $Na_2CO_2/NaHCO_3$, 0.1% sodium azide, and monoclonal mouse NGF antibody (Boehringer Mannheim, Indianapolis, IN) specific for the β-subunit of mouse NGF, at a concentration of 2 µg/ml. After incubation at 37°C for 2 h, the plate was washed with a solution containing 50 mmol/l Tris-HCl, 0.2 mol/l NaCl, 10 mmol/l $CaCl_2$, 0.1% Triton X-100, and 0.05% sodium azide and incubated with blocking solution (coating solution plus 0.5% bovine serum albumin [BSA]) at 37°C for 30 min. After washing, mouse β-NGF standard (Sigma) and samples (100 µl) were added and incubated overnight at 4°C. After washing, 100 µl mouse anti-NGF antibody conjugated to β-galactosidase (Boehringer Mannheim) was added (400 mU/ml), and the plate was incubated for 4 h at 37°C. Color was developed by the addition of substrate solution containing 100 mmol/l HEPES, 150 mmol/l NaCl, 2 mmol/l $MgCl_2$, 0.1% sodium azide, 1% BSA, and 2 mg/ml chlorophenol red-B-D-galactopyranoside. The optical density was measured at 570 nm.

Statistical analysis. Data are expressed as means ± SE. Statistical analysis was performed using Super ANOVA (Abacus Concepts Inc, Berkeley, CA). 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

Effect of 6 and 9 months of STZ-D on body weight and plasma glucose. Body weights were similar in all experimental groups at baseline (not shown). Six and 9 months thereafter, body weights were lower by 33 and 39%, respectively, in STZ-D rats and ND rats ($P < 0.05$) (Table 1). Plasma glucose levels were elevated by 4.8- and 4.9-fold ($P < 0.05$) in the 6- and 9-month STZ-D rat groups, respectively.

Effect of STZ-D on ventricular [^{11}C]HED retention. The effects of 6 and 9 months of STZ-D on retention of [^{11}C]HED in the composite right ventricle and the proximal and distal segments of the left ventricle are shown in Fig. 1*A* and *B*. In 6- and 9-month ND rats, retention of [^{11}C]HED in the composite right ventricle was 23–34% ($P < 0.05$) higher than the corresponding values for the left ventricle, consistent with increased density of sympathetic innervation of the right ventricle compared with the left. No statistically significant differences in [^{11}C]HED retention were found in the proximal versus distal left ventricle of ND rats at either 6 or 9 months. Compared with age-matched ND rats, retention of [^{11}C]HED in the composite right ventricle of STZ-D rats demonstrated a time-dependent decrease of 32% ($P < 0.05$) at 6 months and 46% ($P < 0.01$) at 9 months. In 6-month STZ-D rats, marked differences in regional LV [^{11}C]HED retention were observed. Retention of [^{11}C]HED in the proximal left ventricle had declined by only 9% (NS) compared with ND rats, which contrasted with a 33% ($P < 0.05$) decrease in the distal left ventricle. By 9 months, [^{11}C]HED retention had declined in both the proximal and distal myocardial segment

TABLE 1
Effects of 6 and 9 months of STZ-D on body weight and blood glucose

Group	6 Months			9 Months		
	n	Final weight (g)	Final glucose (mg/dl)	*n*	Final weight (g)	Final glucose (mg/dl)
ND rats	15	592 ± 31	66 ± 9	11	624 ± 24	69 ± 3
STZ-D rats	18	396 ± 20*	315 ± 9*	11	378 ± 18*	340 ± 9*

Data are means ± SE. *P < 0.05 vs. ND.

by 44 and 40%, respectively (P < 0.01), compared with the same segments of ND rats. Therefore, 6 months of STZ-D resulted in distal myocardial sympathetic denervation with sparing of the proximal myocardial segments. By 9 months, sympathetic denervation extended to involve the proximal as well as the distal myocardium.

Effect of 6 months of STZ-D on ventricular norepinephrine content. In both ND and STZ-D rats, norepinephrine content tended to be lower in the distal left ventricle compared with the proximal left ventricle, but the differences did not achieve statistical significance (Table 2). Norepinephrine content, however, was markedly elevated (by up to 89% vs. distal left ventricle in ND rats, P < 0.05) in the right ventricles of both ND and STZ-D rats to levels significantly above the LV segments. No significant differences in norepinephrine content emerged on comparison of ND and STZ-D rats in either the left or right ventricle.

Effect of 6 months of STZ-D on ventricular NGF protein content. The effect of 6 months of STZ-D on left and right ventricular NGF protein content is shown in Fig. 2. In ND rats, no statistically significant differences in NGF protein content were found in any ventricular region. In 6-month STZ-D rats, NGF content decreased by 52 and 82% in the proximal and distal left ventricle, respectively (P < 0.05 and P < 0.01 versus corresponding segments in ND rats), the NGF content in the distal ventricular segments being significantly lower than in the proximal segments (P < 0.05). NGF content in the right ventricle of STZ-D rats was 49% lower than that of ND rats, but this difference did not quite achieve statistical significance (P = 0.07). Therefore, 6 months of STZ-D resulted in a heterogeneous decline of NGF protein content in the left ventricle, with maximal depletion occurring in the distal left ventricle.

Effect of 9 months of STZ-D on LV norepinephrine levels and NGF protein content. After 9 months of experimental diabetes, LV norepinephrine content had declined by 35% in the distal myocardial segments of STZ-D rats to levels significantly lower than both the proximal and distal segments of the ND animals (Table 3). Norepinephrine also decreased by 28% in the proximal myocardial segments of the STZ-D rats, but the decrease failed to achieve statistical significance (P = 0.1). At 9 months in ND rats, LV NGF protein content was decreased by 27% in the distal compared with the proximal myocardial segments. In STZ-D rats, NGF protein content was decreased by 59 and 61% in the proximal and distal myocardial segments, respectively. The difference in NGF protein content between the proximal and distal myocardial segments of STZ-D rats did not achieve statistical significance.

DISCUSSION

Scintigraphic studies in diabetic subjects with DAN have consistently demonstrated heterogeneous LV sympathetic denervation with preservation of "islands" of innervation in the proximal myocardium (8–13). The etiology of this heterogeneous pattern of denervation is unknown, and an animal model had not been identified. The aims of this study were to determine whether this heterogeneous pattern of sympathetic denervation occurs in the heart of a rat model of diabetes and to explore a potential association with regional fluctuations in myocardial NGF protein. After 6 months, myocardial [^{11}C]HED retention had significantly declined in STZ-D

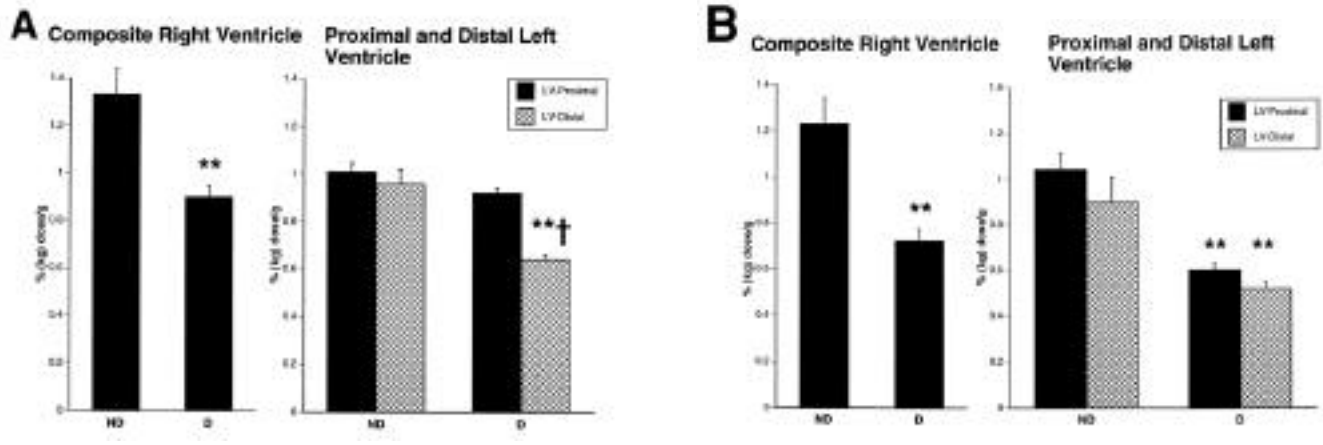

FIG. 1. Effects of 6 (*A*) and 9 (*B*) months of STZ-D on retention of [^{11}C]HED in the composite right ventricle and proximal and distal segments of the left ventricle. Data are means ± 1 SE. **P < 0.01 vs. ND, †P < 0.05 vs. proximal segments of the left ventricle.

TABLE 2
Effects of 6 months of STZ-D on left and right ventricular norepinephrine content

Group	*n*	Right ventricle	Proximal left ventricle	Distal left ventricle
ND rats	10	930 ± 105*	600 ± 51	491 ± 62
STZ-D rats	9	947 ± 65†	711 ± 36	613 ± 68

Data are means ± SE and are given in nanograms per gram of wet weight. *P < 0.05 vs. ND and STZ-D distal left ventricle; †P < 0.05 vs. ND and STZ-D proximal and distal left ventricle.

rats compared with ND rats only in the distal left ventricle. By 9 months, [^{11}C]HED retention deficits extended to involve the proximal myocardium. At 6 months, LV myocardial NGF protein content declined in parallel, with the [^{11}C]HED retention deficits being maximally decreased in the distal left ventricle. At 9 months, norepinephrine levels and NGF protein content were decreased in both the proximal and distal myocardium of STZ-D rats. Therefore, 6 months of STZ-D in the rat results in heterogeneous LV sympathetic denervation, associated with a proximal-to-distal gradient of LV NGF protein depletion. It is tempting to speculate that regional fluctuations of LV NGF protein in the diabetic myocardium contribute to heterogeneous cardiac sympathetic denervation complicating diabetes.

In STZ-D rats at 6 months, [^{11}C]HED retention was decreased selectively in the distal LV segments, but by 9 months, tracer retention deficits had extended to involve the proximal myocardium. These findings are consistent with the pattern of denervation observed in humans, in which the earliest sympathetic deficits are observed in the distal inferolateral wall of the left ventricle and then spread circumferentially and proximally to involve the anterior, inferior, and lateral ventricular walls, reflecting a proximal-distal gradient in the severity of neuropathy (8–13). In patients with severe DAN, [^{11}C]HED retention has been reported to be paradoxically increased in the proximal myocardial segments (12). [^{11}C]HED retention was not increased in the proximal myocardial segments of 6- or 9-month STZ-D rats in this study, which may reflect differences in regional sympathetic tone, neuronal function, or potentially neuronal regenerative responses to nerve injury in the diabetic rodent compared with humans.

Sympathetic neurotransmitter analogs such as [^{11}C]HED and [^{123}I]MIBG are taken up into the neuron by energy-dependent uptake 1 (32,33) and so mark the location of functioning sympathetic nerve terminals. Retention of these tracers in the heart is also dependent on neuronal vesicular storage

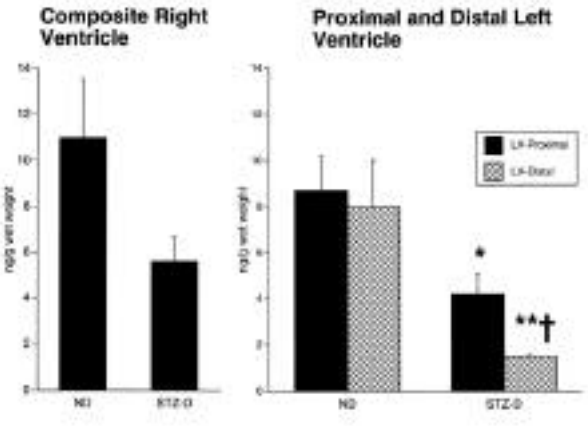

FIG. 2. Effects of 6 months of STZ-D on NGF protein content in the composite right ventricle and proximal and distal segments of the left ventricle. Data are means ± 1 SE. *P < 0.05, **P < 0.01 vs. ND; †P < 0.05 vs. proximal segments of the left ventricle.

(33), and they compete for uptake with endogenous norepinephrine (32). In experimental diabetes, an insulin-sensitive increase in sympathetic activity and elevation of norepinephrine content in the diabetic rat myocardium precedes chronic sympathetic denervation (27–29). The increase of myocardial norepinephrine predominantly reflects increased vesicular storage in the sympathetic nerve terminals, together with an increased concentration of norepinephrine in the synaptic cleft (32,33). In STZ-D rat myocardium at 4–9 weeks, retention of [^{123}I]MIBG is globally decreased at a time when ventricular norepinephrine content is increased (34,35). Furthermore, decreased myocardial [^{123}I]MIBG retention can be prevented by normalization of myocardial norepinephrine content (35). Thus the decrease of myocardial [^{123}I]MIBG retention in acute experimental diabetes may reflect competitive inhibition by norepinephrine for neuronal uptake (32,34), increased [^{123}I]MIBG release (34), or hyperglycemia-induced impairment of neuronal uptake 1 (12), but not neuronal loss. In 6-month STZ-D rats, levels of norepinephrine were not significantly elevated above ND levels, nor did they differ regionally within the left ventricle, and so decreased distal LV [^{11}C]HED retention in the diabetic myocardium at 6 months cannot be ascribed to high levels of endogenous norepinephrine.

Although scintigraphic techniques are able to detect early deficits in sympathetic cardiovascular innervation (8,9,11–13) the precise etiology of these defects is unknown. In humans, small distal deficits of LV [^{11}C]HED retention are reversible

TABLE 3
Effects of 9 months of STZ-D on LV norepinephrine and NGF protein content

Group	*n*	Norepinephrine content		NFG protein abundance	
		Proximal left ventricle	Distal left ventricle	Proximal left ventricle	Distal left ventricle
ND rats	6	694 ± 92	536 ± 56	10.1 ± 0.6	7.4 ± 0.5*
STZ-D rats	6	503 ± 15	351 ± 18†	4.1 ± 0.3‡	2.9 ± 0.4‡

Data are means ± SE and are given in nanograms per gram of net weight. *P < 0.05 vs. ND proximal segments; †P < 0.05; ‡P < 0.01 vs. ND proximal and distal segments.

with the instigation of good glycemic control, indicative of early neuronal dysfunction that precedes complete neuronal loss (36). In this study, a concordant decrease of distal LV [^{11}C]HED retention and NGF protein content was observed in 6-month STZ-D rats, despite unchanged levels of myocardial norepinephrine. After 9 months of STZ-D, however, LV norepinephrine levels, [^{11}C]HED retention, and NGF protein abundance were lower in both the proximal and distal myocardial segments, consistent with sympathetic denervation at 9 months. Neuronal retention of [^{11}C]HED is highly dependent on continuous recycling into and out of the neuron (32,33), but unlike norepinephrine, HED is not metabolized within the neuronal cytosol by monoamine oxidase (32,33) and is thus relatively less dependent on efficient vesicular storage for neuronal retention (33). Therefore, it is tempting to speculate that the time-dependent defects of LV [^{11}C]HED retention observed in STZ-D rats and the dissociation from myocardial norepinephrine content at 6 months could reflect NGF-sensitive stages in sympathetic neuronal dysfunction manifested as early defects of neurotransmitter uptake 1, followed by the development of defective vesicular storage, which may precede complete neuronal loss.

Sympathetic dysinnervation of the left ventricle in STZ-D rats was associated with a concordant decrease in NGF protein content. The etiology of the decline of myocardial NGF protein abundance in experimental diabetes is unknown but may reflect diabetes-induced impairment of NGF synthesis at either a transcriptional or posttranscriptional level. For example, a reduction of NGF mRNA abundance in sensory neuron target tissues has been reported in diabetic rats (23) that correlates with impaired expression of NGF receptors in dorsal root ganglia (24). The effects of diabetes on myocardial NGF gene expression have not been reported, however. Alternatively, in STZ-D rat myocardium, chronically increased oxidative stress (37) may impair NGF protein synthesis (38) or increase NGF protein turnover. Since NGF-sensitive neurons are normally NGF-starved as endogenous NGF levels are limited (22,39) and their NGF receptors are unsaturated (40), small changes in endogenous NGF levels are highly pathophysiologically significant. Although composite LV NGF protein levels have been reported to decrease below ND values after 12 weeks of diabetes in rats (22), this is the first report to demonstrate a proximal-to-distal gradient of NGF protein within the left ventricle after 6 months of experimental diabetes. Further studies are required to explore the pathogenic mechanisms leading to NGF depletion in diabetic myocardium.

Evidence suggests that altered NGF metabolism may play a role in the pathogenesis of diabetic sensory and autonomic neuropathy. In DAN subjects, serum NGF levels are reduced (41), and it has been suggested that NGF autoantibodies may play a role in the development of autonomic dysfunction (42). Decreased skin axon-reflexes, mediated by small sensory fibers, correlate with loss of NGF expression in keratinocytes in patients with early diabetic neuropathy (43,44). NGF binds selectively to the terminal portions of sympathetic and neural crest–derived sensory neurons and, after internalization, is transported by retrograde axonal flow to the cell bodies, a process that is impaired in STZ-D rats (25,26). Ganglionic NGF is required for signal transduction, neurotransmitter synthesis, protein phosphorylation, methylation, and gene expression of Ras-like proteins in sympathetic and sensory neurons (16,17). A recent study in humans suggests that NGF treatment may be effective in the management of sensory neuropathies (45). In experimental diabetes, NGF treatment protects against the development of diabetic sensory neuropathy (46–48) and ameliorates diabetes-induced decreases in neuropeptide levels in vivo (49) and in vitro (50). NGF-treated diabetic rodents retain the ability to respond to noxious thermal stimuli and express normal neuropeptide levels (48). Preclinical studies in diabetic rodents and clinical trials in humans using highly sensitive scintigraphic techniques are required to assess the efficacy of NGF therapy in DAN.

Diabetic autonomic dysfunction and heterogeneous myocardial sympathetic denervation have been implicated as factors contributing to enhanced cardiac risk in diabetic subjects both with and without myocardial ischemia (3,5–7,11–13). Longitudinal studies of DAN subjects have reported the highest mortality rates in severe symptomatic DAN patients with advanced deficits in sympathetic cardiovascular innervation (3,6). Absent heart rate variability in DAN subjects is predictive of LV failure; LV function has been reported to be depressed in 59% of diabetic subjects with DAN compared with only 8% of those without DAN (7). Scintigraphic studies using [^{123}I]MIBG or [^{11}C]HED have demonstrated heterogeneous cardiac sympathetic denervation with islands of persistent proximal innervation (8–13). Coronary flow reserve on adenosine stimulation is also decreased in subjects with advanced DAN, particularly in the islands of persistent innervation (13). Since autonomic imbalance and, in particular, regional cardiac sympathetic hyperactivity increase the risk of cardiac arrhythmias during myocardial ischemia (51) and diabetic patients demonstrate greatly enhanced cardioprotection from β blockade (4), it is tempting to speculate that these islands of persistent adrenergic activity may act as a focus of electrical and chemical instability.

In summary, these studies demonstrate the utility of the STZ-D rat as a model for human cardiac sympathetic denervation complicating diabetes. Distal LV denervation is associated with a proximal-to-distal gradient of myocardial NGF protein depletion. The contribution of regional fluctuations of LV NGF protein to heterogeneous cardiac sympathetic denervation complicating diabetes warrants further investigation.

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