Streptozotocin-induced diabetes differentially affects sympathetic innervation and control of plantar metatarsal and mesenteric arteries in the rat

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Johansen NJ, Tripovic D, Brock JA. Streptozotocin-induced diabetes differentially affects sympathetic innervation and control of plantar metatarsal and mesenteric arteries in the rat. Am J Physiol Heart Circ Physiol 304: H215–H228, 2013. First published November 16, 2012; doi:10.1152/ajpheart.00661.2012.—In humans neural control of arterial vessels supplying skin in the extremities is particularly vulnerable to the effects of diabetes. Here the streptozotocin (STZ) rat model of type 1 diabetes was used to compare effects on neurovascular function in plantar metatarsal arteries (PMAs), which supply blood to skin of hind paw digits, with those in mesenteric arteries (MAs). Twelve weeks after STZ (60 mg/kg ip), wire myography was used to assess vascular function. In PMAs, lumen dimensions were unchanged but both nerve-evoked contractions and sensitivity to α1 (phenylephrine, methoxamine)- and α2 (clonidine)-adrenoceptor agonists were reduced. The density of perivascular nerve fibers was also reduced by ~25%. These changes were not observed in MAs from STZ-treated rats receiving either a low dose of insulin that did not greatly reduce blood glucose levels or a high dose of insulin that markedly reduced blood glucose levels. In MAs from STZ-treated rats, nerve-evoked increases in force did not differ from control but, because lumen dimensions were ~20% larger, nerve-evoked increases in effective transmural pressure were smaller. Increases in effective transmural pressure produced by phenylephrine or α,β-methylene ATP in MAs from STZ-treated rats were not smaller than control, but the density of perivascular nerve fibers was reduced by ~10%. In MAs, the increase in vascular dimensions is primarily responsible for reducing effectiveness of nerve-evoked constrictions. By contrast, in PMAs decreases in both the density of perivascular nerve fibers and the reactivity of the vascular muscle appear to explain impairment of neurovascular transmission.

IN HUMANS ABNORMAL CONTROL of the vasculature has been implicated in the etiology of many diabetes-related complications such as neuropathy and diabetic foot ulceration (41). These changes involve endothelium-dysfunction (30) as well as deficits in both sympathetic nerve-mediated vasoconstriction (see below) and sensory nerve-mediated vasodilation (1). However, the mechanisms whereby diabetes affects sympathetic and sensory nerve regulation of the vasculature are not well understood, although it is believed that diabetes causes degeneration of these nerve supplies (34).

Many clinical studies have evaluated the effects of diabetes on sympathetic nerve-mediated vasoconstriction of arterial vessels supplying plantar skin of the foot. These studies have demonstrated that both type 1 (insulin dependent) and type 2 (noninsulin dependent) diabetic patients with signs of sensory and/or autonomic neuropathy (e.g., orthostatic hypertension) have increased skin blood flow under basal conditions (2, 33) and attenuated reductions in skin blood flow (i.e., vasoconstriction) in response to sympathetic arousal [e.g., produced by mental arithmetic (2) or postural stimuli (29)]. Importantly, deficits in sympathetic vasoconstriction of arterial vessels supplying plantar skin can be demonstrated in both type 1 and type 2 diabetic patients at an early phase of diabetic neuropathy (31, 35), perhaps suggesting that sympathetic neurons supplying these vessels are particularly vulnerable to the effects of diabetes. Furthermore, it has been suggested that reduced sympathetic nerve-mediated vasoconstriction of these vessels is an early change that contributes to the etiology of microangiopathy in skin of the feet (7). In contrast, although type 1 diabetic patients with orthostatic hypertension had increased basal blood flow in the superior mesenteric artery, posturally evoked reductions in blood flow in this vessel were not impaired (26), indicating that sympathetic control of arterial vessels in the splanchnic vascular bed was not detectably changed. These findings may indicate that diabetes differentially affects sympathetic neurons supplying arterial vessels in different vascular beds.

There are only a small number of studies that have investigated the effects of diabetes on sympathetic neural control of arterial vessels in animals. Most of these studies have used the streptozotocin (STZ)-treated rat model of type 1 diabetes and have investigated effects in the rat tail artery, a cutaneous thermoregulatory vessel with a function similar to that of digital arteries in man, and mesenteric arteries (MA). In tail artery, Hart et al. (11) reported that 8 wk of diabetes produced a small reduction in sympathetic nerve-mediated vasoconstriction, whereas Weber and MacLeod (42) and Speirs et al. (32) reported that 12 wk of diabetes did not reduce sympathetic nerve-mediated vasoconstriction. In the perfused mesenteric arterial bed, diabetes reduced sympathetic nerve-mediated pressor responses after 12 wk of diabetes (27), but this effect was not present after 8 wk (28). In mesenteric arteries, it is not known if the decrease in the sympathetic nerve-mediated pressor response produced by diabetes is associated with degeneration of the sympathetic nerve supply, but in tail artery the perivascular sympathetic innervation is not affected by 12 wk of diabetes (32).

In the present study we used the STZ-treated rat model of type 1 diabetes to test the hypothesis that sympathetic control of planter metatarsal arteries (PMA), which supply blood to skin of the hind paw digits, is particularly vulnerable to the effects of diabetes. In this vessel, we investigated whether diabetes modified sympathetic neurovascular transmission and whether the contributions of the cotransmitters, norepinephrine and ATP, to transmission were changed. We also assessed if...
any of the effects of diabetes could be attributed to changes in reactivity of plantar metatarsal arteries to $\alpha$-adrenoceptor and P2X1-purinoceptor agonists or the effectiveness of the neuronal norepinephrine transporter (NET). In addition, the effects of diabetes on peptidergic sensory nerve-mediated vasodilation and the perivascular innervation of planter metatarsal arteries were determined. In these arteries, the effects of insulin treatment were investigated to confirm that any observed effects were due to diabetes and not to the direct toxic effects of STZ treatment. For comparison, the effects of diabetes on sympathetic and peptidergic sensory nerve-mediated control and innervation of small mesenteric arteries supplying the ileal segment of the gastrointestinal tract were investigated.

**MATERIALS AND METHODS**

All experiments were approved by the Animal Ethics Committees at the University of New South Wales (January 2010-December 2010) and the University of Melbourne (January 2010-March 2012) and conformed to the Australian code of practice for the care and use of animals for scientific purposes. Male Wistar rats, 8–10 wk old, were purchased from Animal Resources Center (Canning Vale, WA, Australia). The rats were treated intraperitoneally with 60 mg/kg STZ dissolved in 50 mM citrate buffer (pH 4.5). Age-matched control animals were treated with an equivalent volume of citrate buffer.

Blood glucose levels were measured 5–7 days postinjection using a glucose meter (Accu-Check Performa; Roche Diagnostics Australia Pty) to confirm that the rats were diabetic (>15 mmol/l glucose). Blood glucose levels and body weights were monitored weekly thereafter until they were terminated 12 to 13 wk after the induction of diabetes. Diabetic animals were left untreated or were treated with a low (~1 unit/day) or high dose (~4 units/day) of insulin delivered by sustained-release implants (Linplant, Linshin Canada) inserted subcutaneously beneath the nuchal skin. At 1 and 7 wk after the induction of diabetes, the low-dose group received half a Linplant pellet and the high-dose group received two Linplant pellets. Animals were maintained on a 12-h:12-h light/dark cycle and were provided with food and water ad libitum.

At termination the animals were deeply anesthetized with isoflurane and exsanguinated by cutting the carotid arteries. To assay long-term glycemic control, ~1 ml of blood was collected in EDTA tubes from the STZ-treated rats receiving insulin and their controls to determine the glycated hemoglobin levels by HPLC (CLC330 GHb Analyzer; Primus, Kansas City, MO). The five PMAs branch from the plantar arch (at the distal end of the median plantar artery) and the first two PMAs or second-order MAs were dissected and placed in physiological saline containing (in mM) 133 NaCl, 4.7 KCl, 2.0 CaCl$_2$, 1.2 MgCl$_2$, 1.3 Na$_2$HPO$_4$, 16.3 Na HCO$_3$, and 7.8 glucose. This solution was bubbled with carbogen (5% CO$_2$-95% O$_2$). On each experimental day tissue was collected from both a control and a STZ-treated rat, and the experiments described below were performed in parallel.

**Wire Myography**

Two four-channel myographs (Multi Myograph model 610M; Danish Myo Technology, Aarhus, Denmark) were used to record mechanical responses of the arteries. In each device, two artery segments (~1.5 mm in length) from a control and an STZ-treated rat were mounted isometrically between two stainless steel wires (40 $\mu$m diameter). Arterial segments were bathed in 6 ml of physiological saline that was continuously bubbled with carbogen and maintained at 36° to 37°C. (43). The basal conditions were normalized by gradually stretching the vessel in small steps until the effective transmural pressure (ETP) calculated using Laplace’s equation [transmural pressure = wall tension/[(internal circumference/2$\pi$), where wall tension = force/2 $\times$ vessel segment length] was 13.3 mN/mm$^2$ (100 mmHg) (23). For PMAs, the ETP was then adjusted to 90% of that determined at 13.3 mN/mm$^2$ (23). For MAs, the ETP was set initially at 13.3 mN/mm$^2$. The vessels were then left to equilibrate for at least 30 min. After this period, the basal ETP in MAs stabilized at ~6.5 mN/mm$^2$ (~50 mmHg) and in PMAs at ~10.0 mN/mm$^2$ (~75 mmHg). Under these conditions, MAs (23) and PMAs (Fig. 1) are at the peak of their length-tension relationship. Because diabetes has been reported to change the diameter of MAs (43), the responses to nerve stimulation and to agonists were also converted to ETP to account for any changes in vascular dimensions.

After equilibration, all tissues were exposed to three (PMAs) or four (MAs) applications of phenylephrine (PE; 3 $\mu$M; Sigma-Aldrich, Castle Hill, Australia). The first two applications of PE were for ~4 min and confirmed the viability of the vessels. After the third addition of PE, when the contraction had plateaued, relaxation to carbachol (0.1 and 1 $\mu$M added cumulatively; Sigma-Aldrich) was determined to assess the function of the endothelium. In MAs, when the contraction to the fourth application of PE had plateaued, the relaxation to capsaicin (0.01 and 0.1 $\mu$M added cumulatively; Sigma-Aldrich) was determined to assess the vasodilator effect of activating the perivascular peptidergic primary afferent axons. In MAs, after washout of the forth application of PE, capsaicin (1 $\mu$M) was applied for 10 min to prevent the inhibitory effects of activating the peptidergic primary afferent axons (14). After washout of capsaicin, the MAs were left for a further 30 min before starting the experiments described below.

**Contractions to Neural Stimulation**

Tissues mounted in one four-channel myograph were used to assess contractions to electrical stimulation of the perivascular axons. Electrically induced contractions were obtained with a low-intensity current (100–500 nA) delivered through bipolar stainless steel electrodes (1 mm diameter) from a constant current source (Stat-Pak, Grass Instruments, West Warwick, RI). Each stimulus was repeated 10 times at a frequency of 5 Hz.

**Fig. 1.** The relationship between the internal circumference and the increase in wall tension produced by raising the K$^+$ concentration to 60 mM in plantar metatarsal arteries (PMAs). To construct this relationship, PMAs from 4 animals were set up as described in the MATERIALS AND METHODS, and, after equilibration for 30 min, the level of basal effective transmural pressure was sequentially adjusted to 2.7, 5.3, 8.0, 10.6, and 13.3 mN/mm$^2$ (i.e., 20, 40, 60, 80, and 100 mmHg), with responses to 60 mM K$^+$ determined at each pressure. On the x-axis the internal circumference at each pressure is expressed as a proportion of that at 13.3 mN/mm$^2$ (IC/IC$_{13.3}^\text{3}$). Each point represents the mean of both internal circumference and wall tension measures with X- and Y-SE bars, respectively. In these experiments, the $\alpha$-adrenoceptor antagonists, prazosin (10 nM) and idazoxan (0.1 $\mu$M), were applied to the tissues to prevent the contractile effects of norepinephrine released from the nerve terminals by K$^+$-induced depolarization.
erical stimuli (15 V, 0.2 ms pulse width) were generated by a four-channel stimulator (EXP-ST-CH4; Experimetia, Balatonfüred, Hungary) and delivered via platinum plate electrodes mounted either side of the tissue. In preliminary experiments, it was established that contractions to these stimuli were blocked by tetrodotoxin (0.5 µM), confirming that they are due to action potential-evoked release of neurotransmitter from the perivascular axons. To construct frequency response curves, the PMAs were stimulated with 25 pulses at 0.1, 0.3, 0.5, and 1.0 Hz, whereas the MAs were stimulated with 100 pulses at 1, 2, 3, 5, and 10 Hz. These different stimulus parameters have been selected because PMAs are more responsive to electrical stimulation of their perivascular nerves than MAs, which are only weakly activated by trains of stimuli at 1 Hz (cf. Figs. 3A and 8A). In PMAs from the STZ rats that received no insulin support and their controls, the effects of a 10-min application of capsaicin (1 µM) on contractions to 10 pulses at 1 Hz were then assessed. After washout of capsaicin tissues were left for 30 min before any further assessments.

To assess the effects of neurotransmitter antagonists, the responses of PMAs to 100 stimuli at 1 Hz, and of MAs to 20 stimuli at 10 Hz, were compared before and during the application of the antagonists; the antagonists were in contact with the tissue for at least 20 min before changes to the contractions were determined. For PMAs, the effects of the α1-adrenoceptor antagonist prazosin (10 nM; Sigma-Aldrich) were assessed on one vessel segment and the effects of the α2-adrenoceptor antagonist idazoxan (0.1 µM; Sigma-Aldrich) were assessed on the other. The effects of these antagonists were tested because in other arteries supplying blood to skin both α1- and α2-adrenoceptors contribute to neurovascular transmission (19) and the concentrations used are 10–50 times higher than those corresponding to the pA2 values for prazosin at α1-adrenoceptors (see Ref. 18) and idazoxan at α2-adrenoceptors (see Ref. 6). Subsequently, both α-adrenoceptor antagonists were combined to assess the effects of blocking of all α-adrenoceptors. In addition, in a separate group of PMAs from the STZ rats that received no insulin support, the effects of the P2X1-purinoceptor antagonist NFI449 (10 µM; Tocris Bioscience, Bristol, UK) were assessed. For MAs, where neurotransmission is mediated via α1-adrenoceptors and P2X-purinoceptors (17), the effects of prazosin (10 nM) were assessed in one vessel segment and those of the P2-purinoceptor antagonist suramin (0.1 mM; Sigma-Aldrich) were assessed in the other. Subsequently, both antagonists were combined to assess the effects of blocking of both α1-adrenoceptors and P2-purinoceptors.

Contractions to Chemical Stimuli

Tissues mounted in the other four-channel myograph were used to assess reactivity to exogenously applied agents. For both PMAs and MAs, cumulative concentration-response curves were constructed for the α1-adrenoceptor agonist, PE (0.01–100 µM). To determine the effects of blocking NETs, concentration-response curves for PE were obtained in the absence and in the presence of desmethylimipramine (DMI; 30 nM; Sigma-Aldrich). In PMAs, cumulative concentration-response curves were also acquired for the α1-adrenoceptor agonist methoxamine (0.01–100 µM; Sigma-Aldrich), which is not a substrate for NET (38), and the relatively selective α2-adrenoceptor agonist clonidine (0.001–3.0 µM; Sigma-Aldrich). In PMAs from the STZ rats that received no insulin support and MAs, reactivity to α-β-methylene ATP (0.5–1 µM; Sigma-Aldrich) was assessed. At the end of these experiments, contractions to depolarization of the smooth muscle with physiological saline containing 60 mM K+ (equimolar substitution of KCl for NaCl) were assessed. Before the K+ concentration was raised, prazosin (10 nM) and idazoxan (0.1 µM) were applied to the tissues to prevent the contractile effects of norepinephrine released from the nerve terminals by K+-induced depolarization.

Immunohistochemistry

PMAs and MAs were fixed at their in vivo length in Zamboni’s Fixative overnight at 4°C. The next day tissues were washed in dimethyl sulfoxide (3 × 10 min) to allow for better antibody penetration and then in PBS (pH 7.1–7.2; 3 × 10 min). Tissues were stored in PBS containing 0.1% (wt/vol) sodium azide at 4°C. Arteries were blocked for 1 h at room temperature in 10% normal horse serum in PBS containing 1% (vol/vol) Triton X-100 (Sigma-Aldrich) and then incubated overnight at 4°C in antibody diluent containing mouse anti-tyrosine hydroxylase (TH) antibody (1:1,000; Cat. No. 22941; ImmunoStar, Hudson, WI) and goat anti-calcitonin gene related peptide (CGRP) antibody (1:1,000; Cat. No. 1720-9007; Biogenesis, England, UK). In addition, separate tissues were treated with the pan-neuronal labels rabbit anti-protein gene product 9.5 (PGP9.5; 1:1,000; Cat No. RA95101; Ultraclone, England, UK) or mouse anti-β-tubulin III (1:750; Cat. No. MMS-435P; Covance, Princeton, NJ). Tissues were washed with PBS (3 × 10 min) and incubated at room temperature for 1 h in antibody diluent containing fluorescent secondary antibodies raised in donkey (Molecular Probes), Alexa Fluor 564 anti-mouse (1:500), Alexa Fluor 647 anti-sheep (1:500), and Alexa Fluor 488 anti-rabbit (1:1000). Tissues were again washed with PBS (3 × 10 min) and coverslipped in fluorescence mounting medium (Dako Australia, Campbellfield, Vic, Australia). Z-stacks of the fluorescent images through the entire adventitial thickness were collected using a Zeiss Pascal confocal microscope system. For these images the laser power was set between 10% and 15% and the excitation/emission wavelengths were 488 nm/band pass 505–530 nm for Alexa Fluor 488, 561 nm/band pass 575–615 nm for Alexa Fluor 594, and 633 nm/long pass 650 nm for Alexa Fluor 647. Collection of Z-stacks through the full thickness of the adventitia (15–25 µm) took 8–12 min, and each region of the artery was only imaged once to minimize the effects of bleaching. The specificity of secondary antibodies was tested with omission of the primary antibodies, which always resulted in no immunostaining.

Data Analysis

The output from the myographs was recorded and analyzed using a PowerLab data acquisition system and the program Chart (ADInstruments, Bella Vista, NSW, Australia). The amplitudes of contractions to trains of electrical stimuli and to contractile agents were measured and converted to ETP. The EC50 values for the α-adrenoceptor agonists were determined by fitting the concentration response data to the Hill equation using the curve fitting functions in Igor Pro (Wavemetrics, Lake Oswego, OR) and are presented as their negative logarithm (pEC50). All statistics were performed using SPSS 19 (IBM Corp, Armonk, NY). The stimulus frequency response curves were compared by repeated-measures ANOVA with a single independent variable for between-group comparisons. Other comparisons were made with unpaired t-tests or Mann Whitney U-tests if the variances were not homogeneous (indicate by Levene’s tests). When multiple pairwise comparisons were made in individual tissues, the P values were adjusted using the false discovery rate procedure (5). Data are presented as means and SE or median and interquartile range (IQR) if comparisons were made with Mann Whitney U-tests. P values <0.05 were considered to indicate significant differences. Unless otherwise indicated in the text, P values were obtained using unpaired t-tests. In all cases, n indicates the number of animals studied.

The density of the immunolabeled axon plexus was quantified using maximum intensity Z-projection images collected with ×20 (MAs; pixel size 1.01 µm2) or ×40/×63 objectives (PMAs; pixel size 0.38 µm2/0.24 µm2, respectively). This was done by collecting two line profiles at the same points on all images (1 placed in the upper half and the other in the lower half of the image) using the program ImageJ (National Institute of Health, Bethesda, MD). After the mean maximum background value (determined from 10 points on the image

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where there was no detectable fluorescence) was subtracted, the number (intercept number) and widths (intercept widths) of the regions along the line profiles with pixel values >0 (see Fig. 3, A and B) were determined using an in-house Igor Pro routine. The percent area of the vessel surface covered by the immunolabeled nerve plexus in each image was also measured using ImageJ. For images of TH-immunoreactivity (TH-IR), the mean value of the peaks along the line profiles was measured to ascertain if there was a difference in the intensity of fluorescence of the intercepts (nerve bundles). The integrated TH-IR fluorescence for the entire vessel surface in each image was also measured after subtraction of the mean background level (determined at 10 points on the image where there was no detectable fluorescence) and this was normalized to 100 μm² of vessel surface. For CGRP immunoreactive (CGRP-IR) axons in both PMAs and MAs, the numbers of fluorescent intercepts were determined along three lines placed at the same points (1 placed in the upper half, 1 in the middle, and the other in the lower half of the image) on images collected with a ×20 objective. Measures of intercept number along the line profiles were calculated per 100 μm. For each measure, one-sample t-tests were used to determine whether the measures in arteries from STZ-treated animals expressed as a percentage of those in the control tissues collected on the same day (and processed in parallel) differed significantly from 100%. For each animal and vessel type, the measures were the average obtained from at least three image Z-stacks.

RESULTS

Animals

When assessed 5–7 days postinjection, the STZ rats had blood glucose levels in excess of 20 mmol/l and the rats receiving no insulin (STZ-NI) and those receiving a low dose of insulin (STZ-LI) maintained levels >20 mmol/l. In contrast, the STZ rats receiving a high dose of insulin (STZ-HI) maintained blood glucose levels <15 mmol/l. At termination, STZ-NI rats had little net body weight gain, whereas STZ-LI and STZ-HI rats had greater body weight gain, but this was still smaller than that of their controls (Table 1). However, body weight gain in STZ-HI rats was greater than in STZ-LI rats (Table 1). At termination the blood glucose levels in STZ-NI and STZ-LI rats were greater than those of their controls, but those of STZ-HI rats were less markedly increased (Table 1).

Table 1. Measures of net body weight gain, blood glucose, and percent glycated hemoglobin at termination in control and STZ-treated rats

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Body Weight Gain, g</th>
<th>Blood Glucose Concentration, mmol/l</th>
<th>Percent Glycated Hemoglobin</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>18</td>
<td>156 ± 8</td>
<td>6.5 ± 0.3</td>
<td>0</td>
</tr>
<tr>
<td>STZ-NI</td>
<td>18</td>
<td>12 ± 10</td>
<td>29.4 ± 0.8</td>
<td>0</td>
</tr>
<tr>
<td>STZ-LI</td>
<td>10</td>
<td>126 ± 12</td>
<td>23.9 (23.1–25.2)</td>
<td>14.9 (13.6–15.9)</td>
</tr>
<tr>
<td>STZ-HI</td>
<td>10</td>
<td>240 ± 9</td>
<td>6.3 (5.3–7.3)</td>
<td>5.3 (4.7–5.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>240 ± 9</td>
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<td></td>
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<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
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</tbody>
</table>

Values are means ± SE or medians and interquartile ranges (in parentheses). Statistical comparisons between control and streptozotocin (STZ) groups were made with Student’s unpaired t-tests or Mann Whitney U tests as appropriate. *Significant differences. STZ-NI, STZ rats that received no insulin; STZ-LI, STZ rats that received a low dose of insulin; STZ-HI, STZ rats that received a high dose of insulin.

PMAs

The dimensions and endothelium-dependent relaxations of PMAs were not change by diabetes, but K⁺-evoked contractions were reduced in vessels from STZ-NI rats. The lumen diameter (determined from the measured lumen circumference) and the basal ETP after equilibration for PMAs from STZ-NI, STZ-LI, and STZ-HI rats did not differ from those of their controls (Table 2). Similarly, the percent relaxation of vessels constricted with PE (3 μM) when their endothelium was stimulated with carbachol (0.1 and 1 μM) did not differ between PMAs from each of the groups of STZ rats and their controls (Fig. 2, A–C). The peak increases in ETP produced by depolarization of the vascular muscle with 60 mM K⁺ were smaller in the PMAs from STZ-NI rats than in their controls (Fig. 2D). In contrast, the peak increases in ETP produced by 60 mM K⁺ in PMAs from STZ-LI or STZ-HI rats did not differ from those of their controls (Fig. 2, E and F).

Nerve-evoked increases in ETP were reduced in PMAs from STZ-NI rats. Figure 3 shows stimulus frequency contraction data for PMAs from STZ-NI rats (Fig. 3, A and B), STZ-LI rats (Fig. 3C), and STZ-HI rats (Fig. 3D) together with those for STZ-NI rats. Figure 3 shows stimulus frequency contraction data for PMAs from STZ-NI rats and their controls (Fig. 2, A–C). The peak increases in ETP for PMAs from STZ-NI rats were smaller than control (Fig. 3, A and B). In contrast, stimulus frequency-contraction data for PMAs from STZ-LI and STZ-HI rats did not differ significantly from control (ANOVA between groups P = 0.23 and 0.36, respectively; Fig. 3, C and D).

The effects of capsaicin (1 μM) on responses to 10 pulses at 1 Hz were only determined in PMAs from STZ-NI rats (n = 6) and their controls (n = 6). In both these groups of vessels, the peak amplitudes of the stimulus-evoked contractions measured 3 min after the addition of capsaicin were reduced by about 60% (control, 56 ± 11%; STZ-NI, 64 ± 16%). During the 15-min application of capsaicin the contraction amplitudes returned close to control levels, consistent with this agent activating and desensitizing the peptidergic primary afferent axons. The contractions measured 20 min after washout of capsaicin did not differ significantly from those measured before its addition (percentage of pretreatment contractions: control, 98 ± 12%; STZ-NI, 100 ± 9%; paired t-test; P > 0.8 for both comparisons), indicating that percent glycated hemoglobin levels were significantly higher in both STZ-LI and STZ-HI rats when compared with their controls, but those of STZ-HI rats were less markedly increased (Table 1).

Table 2. The estimated lumen diameter and effective transmural pressure of plantar metatarsal arteries under basal conditions

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Diameter, μm</th>
<th>Basal Transmural Pressure, mN/mm²</th>
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<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>405 ± 19</td>
<td>10.1 ± 0.4</td>
</tr>
<tr>
<td>STZ-NI</td>
<td>10</td>
<td>392 ± 10</td>
<td>9.5 ± 0.3</td>
</tr>
<tr>
<td>STZ-LI</td>
<td>6</td>
<td>392 ± 4</td>
<td>10.2 ± 0.4</td>
</tr>
<tr>
<td>STZ-HI</td>
<td>6</td>
<td>394 ± 8</td>
<td>10.6 ± 0.2</td>
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</tbody>
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Values are means ± SE. Statistical comparisons between control and STZ groups of arteries were made with Student’s unpaired t-tests.
capsaicin desensitization of the peptidergic primary afferent axons did not change the nerve-evoked contractions.

In all groups of PMAs (n = 6 for all STZ and control groups), the contractions to 100 pulses at 1 Hz were reduced by the α1-adrenoceptor antagonist prazosin (10 nM; ~70% blockade) and by the α2-adrenoceptor antagonist idazoxan (0.1 μM; ~30% blockade), and together these agents reduced the contractions by about 85%. The magnitude of the blockades produced by the α-adrenoceptor antagonists did not differ between the arteries from any of the STZ-treated groups and their control groups (P > 0.1 for all comparisons). The P2X1-purinoceptor antagonist NF449 (10 μM) blocked contractions to 100 pulses at 1 Hz by ~20% in arteries from both STZ-NI (19 ± 8%, n = 5) and control rats (18 ± 6%, n = 5; P = 0.92). The effects of NF449 were not tested in arteries from STZ-LI and STZ-HI rats.

PMAs from STZ-NI rats had reduced sensitivity to α-adrenoceptor agonists. Both in the absence and in the presence of the NET inhibitor DMI (30 nM), there was a small but significant decrease in the pEC50 for PE in PMAs from STZ-NI rats compared with their controls (Fig. 3, A and B). This decrease in pEC50 to PE was not observed in PMAs from STZ-LI (Fig. 4, C and D) or STZ-HI rats (-DMI: control 6.03 ± 0.08, STZ-HI 5.98 ± 0.13, P = 0.72; +DMI: control 6.58 ± 0.13, STZ-HI 6.52 ± 0.10, P = 0.74).

The leftward shift in the EC50 for PE produced by blockade of NET with DMI (measured by the EC50 ratio) did not differ between PMAs from any of the STZ-treated groups and their controls (P > 0.2 for all comparisons). Although there was a tendency for the maximum increase in ETP produced by PE to be smaller than control in PMAs from STZ-NI rats (Fig. 4, A and B) and larger than control in PMAs from both STZ-LI (Fig. 4, C and D) and STZ-HI rats (–DMI: control 30.1 ± 1.8 mM/mm2, STZ-HI 33.9 ± 3.1 mM/mm2, P = 0.31; +DMI: control 33.9 ± 3.1 mM/mm2, STZ-HI 36.0 ± 3.2 mM/mm2, P = 0.27), these differences did not reach the level of statistical significance.

In comparison with their controls, PMAs from STZ-NI rats had a reduction in their pEC50 for both methoxamine and clonidine but, in addition, these vessels had a reduction in their maximum contraction to both these agents (Table 3). These reductions in reactivity to methoxamine and to clonidine were not observed in PMAs from STZ-LI rats (Table 3). However, PMAs from STZ-HI rats had an increase in their pEC50 for clonidine, and these vessels also had a trend toward an increased sensitivity to methoxamine (Table 3).

In arteries from STZ-NI rats, the contractions to 100 pulses at 1 Hz were reduced by the α1-adrenoceptor antagonist prazosin (10 nM; ~70% blockade) and by the α2-adrenoceptor antagonist idazoxan (0.1 μM; ~30% blockade), and together these agents reduced the contractions by about 85%. The magnitude of the blockades produced by the α-adrenoceptor antagonists did not differ between the arteries from any of the STZ-treated groups and their control groups (P > 0.1 for all comparisons). The P2X1-purinoceptor antagonist NF449 (10 μM) blocked contractions to 100 pulses at 1 Hz by ~20% in arteries from both STZ-NI (19 ± 8%, n = 5) and control rats (18 ± 6%, n = 5; P = 0.92). The effects of NF449 were not tested in arteries from STZ-LI and STZ-HI rats.

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circular plexus were TH-IR (Fig. 5, G and H). Both the mean peak intensity of the TH-IR fluorescent intercepts along the line profiles and the integrated TH-IR fluorescence were higher than control in vessels from STZ-NI and STZ-LI rats, but not STZ-HI rats (Fig. 5, L and M). However, because TH-IR did not resolve the finest fibers well (Fig. 5, G and H), we chose to use the pan-neuronal markers to assess changes in the density of fibers in the perivascular plexus (Fig. 5, A–F). When compared with that of their controls, the percent area of the PMA surface covered by the axon plexus was reduced in STZ-NI, increased in STZ-LI, and unchanged in STZ-HI rats (Fig. 5I). There was also a ~25% reduction in the frequency of fluorescent intercepts along the line profiles in PMAs from STZ-NI rats, but this change was not seen in PMAs from STZ-LI and STZ-HI rats (Fig. 5f). There was also an increase in the widths of the fluorescent intercepts along the line profiles (Fig. 5K). In addition to TH-IR fibers, a small number of CGRP-IR fibers were also observed within the perivascular plexus (Fig. 6, A and B), but the frequency of CGRP-IR intercepts along the line profiles in vessels from each of the STZ-treated groups did not differ significantly from those in their control vessels (Fig. 6C).

**MAAs**

MAAs from diabetic rats had increased lumen diameters and impaired endothelial vasodilator function but no change in their responses to capsaicin or \( K^+ \). We only studied MAs from STZ-NI rats. After the normalization procedure the lumen diameter was about 20% larger for MAs isolated from the STZ-NI rats compared with their controls (control 382 ± 17 \( \mu \)m, \( n = 8 \); STZ-NI 459 ± 19 \( \mu \)m, \( n = 8 \); \( P < 0.01 \)). However, after equilibration, the basal ETP did not differ significantly between these two groups (control, 7.1 ± 0.3 mN/mm\(^2\); STZ, 6.4 ± 0.4 mN/mm\(^2\); \( P = 0.15 \)). In MAs from STZ-NI rats, the percent relaxation of vessels constricted with PE (3 \( \mu \)M) when the endothelium was stimulated with carbachol (0.1 and 1 \( \mu \)M) was significantly smaller than in control MAs (Fig. 7A). In contrast, the percent relaxation produced when the peptidergic sensory axons were activated with capsaicin (0.01 and 0.1 \( \mu \)M) was

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**Fig. 3.** In PMAs, diabetes reduced nerve-evoked contractions. A: representative traces showing contractions evoked by trains of 25 pulses at 0.1–1 Hz in segments of PMA from a control rat (top) and a STZ-treated rat receiving no insulin (bottom; STZ-NI). B–D: peak increases in effective transmural pressure produced by these stimuli in PMAs from STZ-NI rats (B; \( n = 10 \)) and STZ-treated rats receiving a low dose of insulin (C; STZ-LI, \( n = 6 \)) or a high dose of insulin (D; STZ-HI, \( n = 6 \)) and their controls (\( n = 10 \) for STZ-NI and 6 for STZ-LI and STZ-HI). Data are presented as means and SE. In B, *significant differences between responses of PMAs from STZ-NI and control rats (\( P < 0.05 \); unpaired t-test with \( P \) values adjusted for multiple comparisons using the false discovery procedure).
did not differ between STZ-NI and control MAs (Fig. 7B). The peak increase in ETP produced by application of physiological saline containing 60 mM K\(^+\) also did not differ between these groups of vessels (Fig. 7C).

**Nerve-evoked increases in ETP were reduced in MAs from diabetic rats.** Over the range of stimulation frequencies studied, the nerve-evoked increases in ETP in MAs from STZ-NI rats were smaller than those in MAs from control rats (Fig. 8, Table 3).

**Table 3. Effects of diabetes on concentration-response curves for methoxamine and clonidine in plantar metatarsal arteries**

<table>
<thead>
<tr>
<th></th>
<th>Methoxamine</th>
<th>Clonidine</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>pEC(_{50})</td>
<td>Maximum Contraction, mN/mm(^2)</td>
</tr>
<tr>
<td>Control</td>
<td>6.35 ± 0.08</td>
<td>21.8 ± 0.7</td>
</tr>
<tr>
<td>STZ-NI</td>
<td>6.11 ± 0.06</td>
<td>18.9 ± 1.3</td>
</tr>
<tr>
<td>(P)</td>
<td>&lt;0.05*</td>
<td>&lt;0.05*</td>
</tr>
<tr>
<td>Control</td>
<td>6.11 ± 0.07</td>
<td>26.4 ± 4.8</td>
</tr>
<tr>
<td>STZ-LI</td>
<td>6.16 ± 0.03</td>
<td>25.7 ± 5.5</td>
</tr>
<tr>
<td>(P)</td>
<td>0.50</td>
<td>0.74</td>
</tr>
<tr>
<td>Control</td>
<td>6.14 ± 0.10</td>
<td>27.5 ± 2.7</td>
</tr>
<tr>
<td>STZ-HI</td>
<td>6.36 ± 0.05</td>
<td>33.4 ± 3.1</td>
</tr>
<tr>
<td>(P)</td>
<td>0.08</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Values are means ± SE. Statistical comparisons between control and STZ groups of arteries were made with Student’s unpaired \(t\)-tests. *Significant differences.

Fig. 4. In PMAs, diabetes reduced sensitivity to phenylephrine (PE). A–D: concentration response curves and pEC\(_{50}\)s for PE in PMAs from STZ-treated rats receiving no insulin (A and B; STZ-NI, \(n = 10\)) or a low dose of insulin (C and D; STZ-LI, \(n = 6\)) and their controls (\(n = 10\) for STZ-NI and 6 for STZ-LI) in the absence (A, C) or in the presence of the norepinephrine transporter inhibitor (NET) desmethylimipramine (DMI) (B, D). Data are presented as means and SE. In A and B, *pEC\(_{50}\) values were significantly smaller than control in the PMAs from STZ-NI rats both with and without NET blockade (\(P < 0.05\); unpaired \(t\)-tests).
and B; ANOVA between groups, \( P < 0.05 \)). However, post hoc comparisons between the responses of these groups of vessels at each stimulation frequency did not reach the level of statistical significance. Because the increased dimensions of the MAs from STZ-NI rats might cause the reductions in nerve-evoked increases in ETP, Fig. 8C displays the same data plotted as increases in wall tension (force/2 × vessel segment length) (see Ref. 23). When expressed in this manner the nerve-evoked responses did not differ between MAs from STZ-NI and control rats (ANOVA between groups, \( P = 0.65 \)), indicating that the absolute increase in force generated by nerve stimulation did not differ between these groups of vessels.

In MAs from STZ-NI rats, there was a small but significant decrease in the percent blockade of contractions to 20 pulses at 10 Hz produced by prazosin (10 nM: control 89 ± 2%, \( n = 8 \); STZ-NI 76 ± 5%, \( n = 8 \); \( P < 0.05 \)). However, there was no difference in the percent blockade produced by the P2-purinoceptor antagonist suramin (0.1 mM: control 36%, IQR 31–38%, \( n = 8 \); STZ-NI 43%, IQR 28–66%, \( n = 8 \); Mann Whitney U-test; \( P = 0.63 \)) or by the combination of prazosin and suramin (control 99 ± 1%, \( n = 8 \); STZ 97 ± 2%, \( n = 8 \); \( P = 0.28 \)) between MAs from STZ-NI and control rats.

MAs from diabetic rats had no change in their sensitivity to PE. Both in the absence and in the presence of DMI (30 nM), the pEC50 and maximum increase in ETP to PE did not differ between MAs from STZ-NI and control rats (Fig. 9, A and B). The magnitude of the change in the pEC50 for PE produced by

Table 4. Widths of wholemount planter metatarsal artery segments

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Vessel width, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>284 ± 8</td>
</tr>
<tr>
<td>STZ-NI</td>
<td>7</td>
<td>289 ± 12</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>248 ± 20</td>
</tr>
<tr>
<td>STZ-LI</td>
<td>10</td>
<td>250 ± 17</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>267 ± 15</td>
</tr>
<tr>
<td>STZ-HI</td>
<td>10</td>
<td>280 ± 11</td>
</tr>
</tbody>
</table>

Values are means ± SE.

Fig. 5. In PMAs, diabetes reduced the density of the perivascular nerve plexus and increased the intensity of tyrosine hydroxylase immunoreactivity (TH-IR).

A–F: perivascular plexus revealed with a pan-neuronal marker (A and B, anti-PGP9.5; C–F, anti-β-tubulin III) imaged with a ×63 objective in a PMA segment from STZ-treated rats receiving no insulin (B; STZ-NI), a low dose of insulin (D; STZ-LI), or a high dose of insulin (F; STZ-HI) and their controls (A, C, and E, respectively). Above A and B are shown line profiles measured along the blue lines on the images. G and H: TH-IR perivascular plexus imaged with a ×40 objective in a PMA segment from a control and an STZ-NI rat. I–K: percent area of the vessel surface covered by the immunolabeled nerve plexus (I) and the fluorescent intercept frequency (J) and width (K) along line profiles for artery segments label with a pan-neuronal marker from STZ-NI (\( n = 7 \)), STZ-LI (\( n = 10 \)), and STZ-HI (\( n = 10 \)) rats expressed as a percentage of these measures in paired control tissues. L and M: mean peak value for the TH-IR fluorescent intercepts (L: peak TH) along the line profiles, and the integrated TH-IR fluorescence/100 µm² of vessel surface (M; integrated TH) for artery segments from STZ-NI (\( n = 7 \)), STZ-LI (\( n = 10 \)), and STZ-HI (\( n = 10 \)) expressed as a percentage of these measures in paired control tissues. Data are presented as means and SE. Statistical assessments were made with 1-sample t-tests (*\( P < 0.05 \); **\( P < 0.01 \)). The scale bars in A and G indicate 50 µm and also apply, respectively, in B–F and H.
DMI (measured by the EC50 ratio) also did not differ between these groups of vessels (P = 0.73).

MAs from diabetic rats had no change in their responses to α,β-methylene ATP. The peak increase in ETP to α,β-methylene ATP (0.5 μM) did not differ between MAs from STZ-NI and control rats (control, 12.4 ± 1.7 mN/mm², n = 8; STZ-NI, 11.3 ± 1.1 mN/mm², n = 8; P = 0.59).

MAs from diabetic rats had a reduced density of TH-IR perivascular axon bundles, but this change was associated with an increase in vascular dimensions. The widths of the wholemount MAs from STZ-NI rats were about ~25% larger than those of their controls (control, 322 ± 1 μm, n = 7; STZ-NI, 407 ± 10 μm, n = 7; P < 0.01). In MAs from both STZ-NI and control rats, the perivascular plexus was composed of TH-IR axon bundles of fairly uniform thickness that formed a network pattern across the medial surface (Fig. 10, A and C). In addition, the perivascular plexus of MAs contained a higher density of CGRP-IR fibers than that of PMAs (Fig. 10, B and D; cf. Fig. 6, A and B). Both the percent area of the vessel surface covered by the axon plexus and the frequency of TH-IR intercepts along the line profiles in MAs from STZ-NI rats were ~12% lower than in controls (Fig. 10F), but the widths of the intercepts were similar in both groups of vessels (Fig. 10F). There was also a reduction in the integrated TH-IR fluorescence and tendency for the mean peak intensity of the TH-IR fluorescent intercepts along the line profiles to be less in MAs from STZ-NI rats (Fig. 10F). Relative to control tissues, the frequency of CGPR-IR intercepts along the line profiles in MAs from STZ rats was not significantly different (91 ± 12% of control; 1-sample t-test; P = 0.48).

DISCUSSION

This study provides the first evidence that neurovascular function is differentially affected in arteries supplying blood to
skin of the hind paw digits (PMAs) and to the intestine (MAs). In STZ-NI rats, nerve-evoked increases in ETP were reduced in both PMAs and MAs. The change in neurovascular transmission in PMAs was associated with reduced reactivity to α-adrenoceptor agonists and to high [K\(^+\)], whereas no change in responsiveness to exogenously applied agents was detected in MAs. PMAs from STZ-LI and STZ-HI rats had no change in their nerve-evoked contractions and their reactivity to exogenously applied agents was not reduced, confirming that the changes observed in PMAs from STZ-NI rats were not the result of a direct toxic action of STZ. The frequency of nerve bundles was reduced in the perivascular nerve plexus of PMAs and MAs from STZ-NI rats and in PMAs both the intensity of TH-IR and thickness of the nerve bundles (intercept widths) were increased. Although the density of perivascular nerve bundles was not significantly changed in PMAs from STZ-LI rats, the intensity of TH-IR and the thickness of bundles were increased. These changes were not detected in PMAs from STZ-HI rats.

Previous studies investigating changes in sympathetic neurovascular function in rats with STZ-induced diabetes have not provided any insulin support. As reported by others (8), treatment of the STZ-treated rats with a low dose of insulin that leaves them markedly hyperglycemic improved the overall health of the animals compared with those receiving no insulin support as indicated by the increase in body weight gain. In comparison with those of STZ-LI rats, the terminal glycated hemoglobin levels in STZ-HI rats were reduced by about 50%, indicating effective long-term glycemic control. However, in comparison with control rats, the STZ-HI rats at termination still had elevated levels of glycated hemoglobin and reduced body weight gain.

The effects of STZ-induced diabetes on PMAs have not previously been reported, but they have been described for small MAs. As we observed, there are several reports that diabetes impairs endothelium-mediated vasodilation of MAs (12, 36, 44). Also as we observed, Wigg et al. (43) found that diabetes increased the lumen diameters of MAs, and these investigators demonstrated that this change occurred without alterations in the thickness or the mechanical properties of the vascular wall. A chronic increase in blood flow can increase the lumen diameter of MAs (10), so a possible explanation for change in vessel dimensions observed in STZ-treated rats is an increase in intestinal perfusion to meet the demands produced by diabetes-induced hyperphagia (43). The reported effects of STZ-induced diabetes on the reactivity of MAs to α-adrenoceptor agonists are variable, with sensitivity to these agents being either increased (36) or unchanged (12, 44).

With passive (i.e., unconstricted) diameters of ~400 μm, the size of second-order MAs in control rats is at the upper end of the arterial vessels that produce resistance to blood flow (see Ref. 3). However, because sympathetic nerve activation of MAs in vivo can reduce their internal diameter by 50–70% (9),

![Fig. 8. In MAs, diabetes reduced nerve-evoked increases in effective transmural pressure but did not significantly change these responses when expressed as increases in wall tension. A: representative traces showing contractions evoked by trains of 100 pulses at 1–10 Hz in a segment of MA from a control rat (top) and an STZ-treated rat (bottom). B and C: peak increases in effective transmural pressure (B) or wall tension (C) produced by these stimuli in MAs from control (n = 8) and STZ-treated rats (n = 8). Data are presented as means and SE. In B and C, the ANOVA P values are for between group comparisons. Although the ANOVA indicated that nerve-evoked increases in effective transmural pressure in MAs from STZ-treated rats were smaller than those of their controls, comparisons between the responses of these groups of vessels to each frequency of stimulation did not reach the level of statistical significance.](http://ajpheart.physiology.org/)

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it is suggested that arterial vessels of this size play a more important role in neural regulation of peripheral resistance. Nerve-evoked pressor responses of the perfused isolated mesenteric arterial bed were reduced by 12 wk of diabetes (27). As the basal perfusion pressure was also decreased by diabetes (27), the reduced nerve-evoked pressor responses are possibly explained by the increased lumen dimensions of the resistance arteries in this vascular bed rather than a change in neurovascular transmission. This appears to be the case in the present study because, although the nerve-evoked increases in ETP were reduced in the MAs from STZ rats, the absolute increases in force produced by nerve stimulation in these vessels did not differ from those in control MAs. These findings contrast with those for the increases in ETP produced by 60 mM K+, PE, and α,β-methylene ATP, which did not differ between MAs from STZ and control rats and therefore appear to scale with the change in vascular dimensions.

Like second-order MAs, the PMAs had passive diameters of ~400 μm and it is therefore likely that constriction of these vessels contributes to the neural regulation of blood flow in the hindpaw digits. The dimensions of PMAs from STZ-NI rats were similar to those of their controls, and in these vessels the increases in absolute force produced by nerve stimulation (not shown) were reduced to a similar extent as the nerve-evoked increases in ETP. Therefore, neurovascular transmission was reduced in PMAs from STZ-NI rats. The reduction in neurovascular transmission appears to be accounted for, at least in part, by postjunctional changes in the vascular muscle because these vessels also had reduced sensitivity to α-adrenoceptor agonists with decreases in their pEC50s for PE, methoxamine,
and clonidine and smaller maximum contractions to methoxamine and clonidine. Because the contractions to 60 mM K\(^+\) were also reduced in PMAs from STZ-NI rats, there appears to be a generalized decrease in the reactivity of these vessels to contractile agents. However, we cannot exclude the possibility that the expression of \(\alpha_1\)- and \(\alpha_2\)-adrenoceptors in the vascular muscle of PMAs is reduced by diabetes. Contractions to 1 \(\mu\)M \(\alpha,\beta\)-methylene ATP were not reduced in PMAs from STZ-NI rats, but these responses were considerably smaller than those produced by highest concentrations of \(\alpha\)-adrenoceptor agonists tested or by 60 mM K\(^+\), and perhaps we would have seen a reduction had we used a higher concentration of this agent.

PMAs are likely to be under strong thermoregulatory control like the rat tail artery that supplies blood to the skin of the tail (25). In rat tail artery, 8 wk of STZ-induced diabetes has been reported to produce a small decrease in nerve-evoked contractions (11), whereas another study found no change in nerve-evoked contractions after 12 wk of diabetes (32). In both these studies, the sensitivity to norepinephrine was assessed with the former study reporting no changes (11) and the latter reporting an increased sensitivity to this agent (32). In the study of Speirs et al. (32), the tail arteries from diabetic rats were studied in physiological saline containing a high concentration of glucose (25 mM), and in control vessels bathed in the same solution the sensitivity to norepinephrine was similarly increased. In preliminary studies we assessed the effects of increasing the glucose to 25 mM on reactivity of PMAs to nerve stimulation and to PE, methoxamine, and clonidine and observed no changes. Speirs et al. (32) also reported an increased contribution of purinoceptors to nerve-evoked contractions of tail arteries from diabetic rats. The relative contribution of P2X1-purinoceptors to neurovascular transmission (assessed with NF449) in PMAs from STZ-NI rats was not changed. Furthermore, blockade of \(\alpha_1\)- and \(\alpha_2\)-adrenoceptors with prazosin and idazoxan, respectively, reduced nerve-evoked contractions to a similar extent in PMAs from all the STZ and control groups of rats. In MAs, the blockade of nerve-evoked contractions produced by the P2-purinoceptor antagonist suramin was not changed by diabetes.

PMAs from STZ-LI and STZ-HI rats did not have reduced nerve-evoked contractions and their sensitivity to \(\alpha\)-adrenoceptor agonists was also not reduced. The findings in PMAs from STZ-LI rats, which remained markedly hyperglycemic, may be explained by the improved health of these animals, and it is possible that we would have observed changes had a longer period of diabetes been studied. However, it is also possible that some of the changes observed in the PMAs from STZ-NI rats are the result of the marked reduction in insulin levels rather than hyperglycemia (24) and that these are prevented in the STZ-LI rats. In PMAs from STZ-HI rats, we observed an increase in sensitivity to clonidine and a trend toward an increase in sensitivity to methoxamine. These effects may also be explained by a direct effect of insulin treatment, which has been reported to increase vascular reactivity to \(\alpha\)-adrenoceptor agonists (15).

The effects of STZ-induced diabetes on the sympathetic innervation of arteries have rarely been investigated and where they have been examined no changes have been detected [tail artery (32); cerebral arteries (16)]. In MAs from STZ rats, we observed a \(\sim 12\%\) decrease in both the frequency of TH-IR intercepts along the line profiles of the perivascular plexus and the percent area of the vessel surface covered by the immunolabeled nerve plexus. This reduction in nerve fiber density cannot be attributed to a difference in fixation-induced shrinkage because, as with the estimated lumen diameters of the myograph mounted vessels, the widths of the wholemount MAs from STZ rats were 20–25% larger than those of control MAs. However, as the surface area of the vessels was increased, the total number of nerve bundles supplying a segment of artery was not reduced. Instead, it appears that the number of axon bundles in the perivascular plexus did not increase proportionately with the increase in vascular dimensions. This possibility may explain why the absolute increase in force produced by nerve stimulation did not differ between MAs from STZ and control rats.

In PMAs from STZ-NI rats, where the vascular dimensions were not changed, the \(\sim 25\%\) reduction in the frequency of fluorescent intercepts along the line profiles indicates that the total number of nerve bundles supplying a segment of artery was reduced. In rat tail arteries, a reduction in innervation density due to axon loss is associated with a decrease in the activity of neuronal NET (39). Because the effects of blocking NET on sensitivity to PE did not differ between PMAs from STZ-NI and control rats, perhaps this indicates that the changes in the perivascular plexus are produced by rearrangement of the terminal axons rather than loss of axons. In PMAs from both STZ-NI and STZ-LI rats, the fluorescent intercept widths along the line profiles were larger, indicating thicker axon bundles. As a result, the percent area of the vessel surface covered by the immunolabeled nerve plexus was only reduced by \(\sim 12\%\) in STZ-NI rats, whereas it was increase by \(\sim 21\%\) in STZ-LI rats. The cause of thickening of the axon bundles was not further investigated but it has been reported that STZ-induced diabetes causes swelling of sympathetic axons supplying the intestine (4), corpus cavernosum (22), seminal vesicle (21), and pineal gland (40). In addition to the changed morphology of the perivascular nerve plexus, both the mean peak intensity of the TH-IR fluorescent intercepts along the line profiles and the integrated TH-IR fluorescence were increased in PMAs from both STZ-NI and STZ-LI rats, suggesting an increased content of TH. An increase in the intensity of TH-IR fluorescence has been reported for sympathetic axons supplying the corpus cavernosum (22) and seminal vesicle (21) in STZ rats. The changes in the structure of the axon plexus and in TH-IR suggest that plastic changes are taking place in the sympathetic innervation of PMAs. No changes were detected in the perivascular axon plexus of PMAs from STZ-HI rats, indicating that the effects seen in PMAs from both STZ-NI and STZ-LI rats are most likely explained by hyperglycemia.

Sensory nerve-mediated vasodilation of the perfused isolated mesenteric arterial bed has been reported to be reduced in rats 8 wk after induction of diabetes with STZ (28). However, in the present study, we observed no changes in the peptidergic innervation of either MAs or PMAs. Furthermore, we did not detect changes in the inhibitory effects of activating the perivascular peptidergic axons with capsaicin on PE-constricted MAs, or on nerve-evoked contractions of PMAs, from STZ-NI rats.

Together these findings suggest that sympathetic neurons supplying the PMAs are more markedly affected by diabetes than those supplying the mesenteric arteries. For somatic sensory and motor neurons, it is known that neurons with long
axons (i.e., supplying the limbs) are most susceptible to diabetes-induced damage (1). Little is known about the length dependence of the effects of diabetes on autonomic neurons. However, because deficits in sympathetic nerve-mediated vasconstriction of arteries supplying skin of the hands and feet can be an early sign of diabetic autonomic neuropathy (13, 35), sympathetic neurons with long axons may also be particularly susceptible to the effects of diabetes. In rat tail artery, STZ-induced diabetes is reported to produce a length-dependent increase in the neuronal content of biogenic amines (norepinephrine, adrenaline, serotonin, and dopamine), with the most marked changes occurring in the most distal region of this vessel (20). This length dependence raises the possibility that axonal transport is affected. Previous studies indicated that diabetes affects both anterograde and retrograde axonal transport in the sciatic nerve (37), so in the nerve terminal axons there may be changes in the turnover of proteins required for the normal processing of these biogenic amines (e.g., enzymes and transporters). A change in axonal transport may also explain the increased level of TH-IR detected in PMAs from STZ-NI and STZ-LI rats.

In conclusion, this study demonstrates that the effects of diabetes differ between PMAs and MAs. Although in both vessels there were reductions in nerve-evoked responses, only in PMAs did diabetes appear to affect the sympathetic nerve terminals. In MAs, the observed reduction in innervation density produced by diabetes can be explained by the increased size of these vessels. In PMAs, the reduction in nerve-evoked contractions may also be explained by a decrease in the reactivity of their vascular muscle to α-adrenoceptor agonists. Importantly, in PMAs all effects of STZ-induced diabetes observed in this study were prevented in the rats receiving a high dose of insulin, demonstrating their dependence on hyperglycemia and/or loss of insulin signaling. Therefore, PMAs provide a suitable model to investigate the effects of diabetes on sympathetic vasoconstrictor neurons and neurovascular transmission, and to assess the efficacy of neuroprotective treatments.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS


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