Microvascular Reactivity to Norepinephrine at Different Arteriolar Levels and Durations of Streptozocin-Induced Diabetes

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Microvascular disease is a hallmark of diabetes. Although the etiology of diabetic microangiopathy remains to be elucidated, numerous studies in experimental animals and human diabetic patients have suggested that a change in vascular reactivity to vasoactive agents is a component of the pathophysiology. Most previous studies have utilized indirect methods to study the microcirculation or have conducted studies of responses in large vessels. This study was designed to directly study in an intact microvascular preparation the effects of streptozocin-induced diabetes (STZ-D) on microvascular reactivity. The responses of arterioles in the cremaster muscle of pentobarbital sodium–anesthetized rats to topically applied norepinephrine were measured in STZ-D rats of 2, 4, 8, 16, and 32 wk duration and in age-matched nondiabetic rats. Resting arteriolar diameters and mean arterial pressure were not affected by diabetes. In the STZ-D rats, larger (1A) arterioles were normally responsive after 2, 4, 8, and 16 wk of diabetes compared with nondiabetic rats. In contrast, the smaller 2A and 3A arterioles exhibited hypersensitivity to norepinephrine initially, but responses returned to normal sensitivity as the duration of diabetes progressed to the chronic stage. These results suggest that there are important functional changes in the responses of the microcirculation to norepinephrine that are associated with the development of diabetes and that these changes are anatomically specific and temporally dependent.
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Diabetes mellitus is associated with clinically significant alterations in the microcirculation. Although these alterations have been studied extensively in diabetic human patients (1) and animals with chemically induced or spontaneous diabetes (2), the basic etiology of the microvascular disease remains largely unknown. One theory has attributed certain functional microvascular changes to altered responsiveness of the vascular smooth muscle to circulating vasoactive agents (3,4). To investigate this theory, vascular reactivity to various vasoactive agents during diabetes has been measured in isolated vascular tissue (5–12) and, to a lesser degree, in intact microvascular preparations in situ (13–15). The results obtained in these studies have varied considerably, some reporting increased, some decreased, and some unchanged vascular reactivity associated with diabetes. Many factors such as the particular vessels studied, animal species, diabetogenic agent utilized (alloxan vs. streptozocin [STZ]), and duration of diabetes appear to affect the results.

MacLeod and McNeill (10) recently reported that the duration of the diabetic state had a significant effect on the reactivity of vessels to norepinephrine; they found no change in sensitivity at 7 days, hypersensitivity at 100 days, and a return to normal sensitivity after 180 days of diabetes in aortas from STZ-administered rats. The transient changes in reactivity observed in these studies suggest that profound and complex alterations in vascular function may indeed be developing in the early stages of diabetes and that these changes are not static but rather are modulated by other intrinsic or extrinsic factors that come into play as the disease progresses. Some studies of functional vascular responsiveness in diabetic patients are supportive of this concept (16–18).

One limitation of most of the studies cited above is that vascular responsiveness was either measured in vitro for tissue from large vessels or for whole vascular beds comprised of both macro- and microvascular components. The applicability of these results to the situation that is obtained in intact microvascular vessels might be questioned. This study directly measured in an intact microvascular prepa-

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ration the reactivity to norepinephrine at various stages of STZ-induced diabetes (STZ-D).

RESEARCH DESIGN AND METHODS

Surgical preparation and induction of diabetes. All experiments were performed on male Sprague-Dawley rats anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg i.p.). The rats were maintained in a controlled environment and were provided with food and water ad libitum. Rats in the 50- to 75-g body-weight range were received from the supplier and were allowed to acclimatize to the housing environment for 1 wk before use in any of the acute experiments. During the course of an experiment, anesthetic supplements equal to 20% of the initial dose were administered intraperitoneally as required. To induce diabetes, some rats were given a single intracardiac injection (under light ether anesthesia) of 65 mg/kg STZ (SO130, Sigma, St. Louis, MO) in saline solution acidified to pH 4.5 with citrate. The nondiabetic control rats were injected with saline solution by the same procedure.

The surgical preparation of the cremaster muscle utilized in these studies is a modification of the technique developed by Baez (19) and is described in detail elsewhere (20). Briefly, the right cremaster muscle was exposed by longitudinally cutting the scrotal sac and gently teasing away the testicle, which was then pushed into the abdominal cavity. The cremaster muscle was then cut longitudinally with a small electrocautery along a line opposite to the location of the main supply vessels. The animal was then placed on its back on a heating pad and positioned on a Plexiglas board fitted with a cremaster bath chamber. The cremaster muscle was secured in place over an optical port in the bath chamber with five 4-0 silk sutures placed around the cut muscle periphery, and the chamber was sealed with modeling clay and silicone grease and filled with ~30 ml of a modified bicarbonate-buffered Krebs solution (25.5 mM $NaHCO_3$, 112.9 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2 \cdot 2H_2O$, 1.19 mM $MgSO_4 \cdot 7H_2O$, and 11.6 mM dextrose). Throughout the surgical and experimental procedures, the rectal temperature of the rats was monitored and controlled between 35 and 37°C by adjusting the voltage applied to the heating pad. Back temperature was monitored to ensure that excessive heat (>40°C) was not being applied to the rat. The temperature of the bath solution was also monitored and controlled at 34.5 ± 0.3°C by a feedback system that regulated the current passing through an electrically isolated coil of nichrome wire in the bath chamber. Bath pH was monitored continuously by an indwelling combination electrode, and the bath O_2 and CO_2 tensions (Po_2 and Pco_2) were determined at 15-min intervals with a clinical blood gas analyzer. Bath pH, Po_2, and Pco_2 were controlled around the desired levels (pH 7.4, Po_2 40 mmHg, and Pco_2 35 mmHg) throughout the experiments by varying the rates of bubbling of O_2, CO_2, and N_2 through the bath chamber. The osmolarity of the bath solution was determined periodically to ensure that it remained within the desired range of 280–300 mmol/kg. In a previous study (21), we determined that the cremaster muscle, prepared and maintained as described, has a blood flow per unit weight that is similar to other striated muscle and that the blood flow is not significantly altered by the surgical exposure.

Mean arterial blood pressure was monitored continuously in all rats by a polyethylene cannula in the left femoral artery connected to a pressure transducer (Century Technology CP-1) and chart recorder (Beckman R411). At the conclusion of each experiment, an arterial blood sample was collected from the femoral cannula, and the Po_2 and Pco_2 were measured with a blood gas analyzer (Instrumentation Laboratories 1302). Strict limits were placed on the acceptable mean blood pressure (not <80 mmHg), Po_2 (not <80 mmHg), and Pco_2 (between 35 and 45 mmHg). If any of the experimental data for these parameters fell outside of the designated ranges, the rat was considered to be physiologically unacceptable, and the data from that rat were not included in the results. At the termination of each experiment, a blood sample was also collected for determination of the plasma glucose concentration by the *o*-toluidine colorometric method.

Data collection and experimental protocol. To visualize the microcirculation, the rat still on the board was placed on the movable stage of a trinocular microscope (Leitz Dialux). The cremaster muscle was transilluminated with a 100-W tungsten-halogen lamp, and a 420-μm narrow band-pass filter was placed in the illumination pathway to enhance image contrast between vessels and surrounding tissue. The image from the vertical microscope port was projected onto a 1-inch vidicon television camera (RCA model TC1005) for viewing on a 13-inch diagonal television monitor (RCA model TC1214) and recorded on videotape (Sony AV3650) for off-line analysis. A ×10 long-working-distance objective and ×16 projection eyepiece lens were utilized, yielding a total magnification on the television monitor of about ×1500. A video clock image was superimposed on the display to facilitate experiment timing.

The internal diameters of arterioles within the cremaster muscle were determined with an image-shearing monitor system (Instrumentation for Physiology and Medicine, model 907) connected in parallel with the video monitor. The output from the image-shearing monitor was continuously recorded on a strip-chart recorder calibrated to read directly in micrometers before each experiment with the image of a stage micrometer. Mean arterial pressure, bath pH, and rectal, back, and bath temperatures were also recorded continuously throughout the experiments.

Rats were divided into two groups for this study: nondiabetic control rats (designated ND) and STZ-D rats. To investigate the time course of the development of the observed responses, STZ-D rats were studied at various durations after the induction of diabetes (2, 4, 8, 16, and 32 wk) and are designated STZ-2, STZ-4, etc. The ND rats used for comparison were age matched to the STZ-D rats and are identified as ND-2, ND-4, etc. The purpose of the experiments was to determine the effects of diabetes on the reactivity of cremaster muscle arterioles to topically applied norepinephrine. To measure this reactivity, concentration-response curves were obtained for cremaster arteriole diameters in each rat by the addition of between 5 and 7 concentrations of norepinephrine bitartrate (A9512, Sigma) directly into the cremaster bath. The responses of 1st-branching-order (1A), 2nd-branching-order (2A), and 3rd-branching-order (3A) arterioles were investigated. Usually, two vessels of different branching orders were studied in

each animal. However, in the older animals (ND-32 and STZ-32), the thickness of the cremaster prevented accurate measurement of the diameter of 1A arterioles, so no data for the 1A vessels are presented for the 32-wk rats. The experimental protocol for each norepinephrine concentration was as follows: a 5-min period for measurement of control vessel diameters, addition of norepinephrine to the bath to yield a specific molar concentration, a 10-min response period in which the change in diameter of a given arteriole was measured, and a 15- to 20-min recovery period during which the cremaster bath was drained and refilled repeatedly with fresh Krebs solution. After recovery of the vessel diameter to the original control value, the protocol was repeated for the next higher dose of norepinephrine.

Data analysis. The data for arteriolar diameters were normalized by expressing the data as a percentage of the average value during the control period immediately preceding the application of each dose, and a percent constriction was determined. For example, an arteriole that changed diameter from 50 to 40 μm in response to a given dose would be said to have a 20% constriction for that dose. Individual concentration-response curves were constructed for each vessel in each experiment, and the concentration that produced 50% of the maximum constriction (ED_{50}) was graphically determined. These values were converted to pD_2 values ($pD_2 = -\log ED_{50}$), which indicate the level of sensitivity of the vessels to norepinephrine (22). An analysis of variance was carried out to determine if the mean pD_2 values obtained for the arteriolar responses at the different durations of diabetes were statistically different. If the analysis of variance indicated a statistically significant difference among the sample means, Duncan's new multiple-range test for multiple comparisons was applied to determine which of the sample means were different. A significance level of 0.05 was used for all tests.

RESULTS

A total of 163 experiments were included in this study. Table 1 shows the control data (body weight, mean arterial pressure, and plasma glucose) for all of the rats studied. The growth curve for the STZ-D rats was depressed, leading to body weights for the STZ-8, STZ-16, and STZ-32 rats that were significantly less than the age-matched ND rats. Plasma glucose values averaged ~125 mg/dl in the ND rats and were significantly elevated to ~500 mg/dl in all of the STZ-D rats. Mean arterial pressure was ~100 mmHg in all rats and was not significantly different in any of the diabetic groups compared with age-matched controls.

TABLE 1
Data on nondiabetic (ND) and streptozocin-induced diabetic (STZ) rats over time (2, 4, 8, 16, or 32 wk)

Group	*n*	Body weight (g)	MAP (mmHg)	Plasma glucose (mM)
ND-2	18	118 ± 7.1	94 ± 3.7	6.8 ± 0.6
STZ-2	14	115 ± 7.9	98 ± 4.1	27.4 ± 2.3*
ND-4	20	165 ± 8.3	103 ± 3.9	7.3 ± 1.4
STZ-4	19	128 ± 12.4	100 ± 3.5	28.5 ± 2.8*
ND-8	18	198 ± 12.7	102 ± 2.9	7.5 ± 1.3
STZ-8	13	135 ± 20.2*	96 ± 4.1	28.0 ± 2.1*
ND-16	15	246 ± 15.2	97 ± 4.3	6.6 ± 1.8
STZ-16	19	141 ± 25.5*	99 ± 3.3	27.3 ± 2.9*
ND-32	15	297 ± 23.3	101 ± 3.7	7.8 ± 1.6
STZ-32	12	150 ± 31.7*	104 ± 3.4	27.2 ± 3.4*

Data are means ± SD. MAP, mean arterial pressure during first control period.
*$P < 0.05$ vs. age-matched ND rats.

TABLE 2
Arteriole diameters of nondiabetic (ND) and streptozocin-induced diabetic (STZ) rats over time (2, 4, 8, 16, or 32 wk)

Group	*n*	Diameter (μm) 1A	2A	3A
ND-2	18	93 ± 5.4 (11)	47 ± 4.3 (11)	26 ± 2.7 (12)
STZ-2	14	97 ± 5.9 (12)	45 ± 3.9 (9)	27 ± 3.3 (10)
ND-4	20	105 ± 6.3 (8)	49 ± 4.1 (14)	29 ± 2.7 (11)
STZ-4	19	94 ± 6.0 (12)	47 ± 3.5 (9)	29 ± 2.2 (9)
ND-8	18	113 ± 5.8 (12)	47 ± 3.7 (11)	26 ± 3.1 (9)
STZ-8	13	94 ± 5.3 (7)*	43 ± 4.4 (8)	30 ± 3.4 (9)
ND-16	15	123 ± 6.6 (7)	53 ± 3.1 (11)	27 ± 3.3 (8)
STZ-16	19	98 ± 6.1 (8)*	45 ± 4.3 (12)	28 ± 3.9 (11)
ND-32	15		52 ± 3.7 (9)	31 ± 3.4 (8)
STZ-32	12		47 ± 4.1 (7)	31 ± 4.1 (9)

Data are means ± SD. Numbers in parentheses after each diameter are the number of vessels of each branching order studied in each group. 1A, 2A, and 3A indicate 1st-, 2nd- and 3rd-branching-order arterioles, respectively.
*$P < 0.05$ vs. age-matched ND rats.

Table 2 presents the resting control diameters for 1A, 2A, and 3A arterioles for all rats. In general, the diabetic state did not appear to have any significant overall effect on resting arteriolar diameter, although the 1A arterioles were significantly smaller in the STZ-D rats at 8 and 16 wk of diabetes compared with diameters in the age-matched ND rats. However, it is questionable whether a direct comparison of vessel diameters between rats of significantly different size is appropriate. In general, the arterioles were larger in diameter in the larger rats, and it is therefore difficult to tell whether the differences in diameter observed between the STZ-D and ND rats was due to a diabetes-related change in resting vascular tone or simply due to the fact that the STZ-D animals were smaller in size. If these diameters are adjusted to compensate for differences in body weight, the arteriolar diameter differences are no longer statistically significant.

Responsiveness of different arteriolar segments to topically applied norepinephrine was determined by measuring the changes in diameter of arterioles by branching order in both ND and STZ-D rats. The diameter dose-response data obtained for the 1A arterioles are shown in Fig. 1. The ordinate is percent constriction plotted versus the molar dose of norepinephrine on the abscissa (dose range 10^{-9}–10^{-4} M). Dose-response curves were obtained in diabetic rats at various times after STZ injection, and these curves are plotted with different symbols in Fig. 1. The percent constriction shown for each dose in each diabetes-duration group is the mean value for all vessels of a given branching order studied in that group. The number of rats and vessels utilized to calculate each point in Fig. 1 is indicated in Table 2. Norepinephrine dose-response curves were also obtained for vessels of different branching order in ND rats that were age-matched to STZ-D rats. Only one

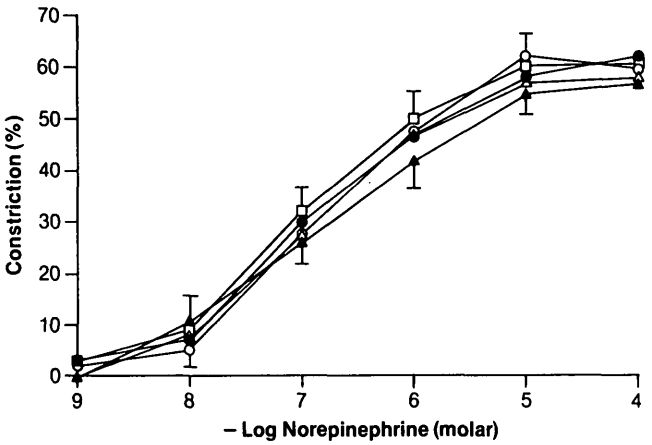

FIG. 1. Norepinephrine dose-response relationships for 1st-branching-order arterioles. Ordinate is percent constriction plotted vs. molar dose of norepinephrine as negative log on abscissa (e.g., 7 indicates dose of 10^{-7} M). Responses are shown for streptozocin-induced diabetic rats 2 (●), 4 (△), 8 (▲), and 16 (□) wk after induction of diabetes. For reference, response obtained in 2-wk age-matched nondiabetic rats (○) is shown. Dose responses were also obtained for age-matched nondiabetic rats at 4, 6, 8, and 16 wk, but these were not significantly different from those at 2 wk. *Bars*, representative standard deviations.

of the average dose responses for ND rats (ND-2) is shown in Fig. 1. Even though the curves for the other ND rats are not shown, note that all statistical comparisons were made to the age-matched ND rats, i.e., STZ-2 compared to ND-2, STZ-4 compared to ND-4, etc. The norepinephrine dose-response relationships obtained for the 2A and 3A arterioles are shown in Figs. 2 and 3.

To assess the responsiveness and sensitivity of the arterioles, maximum percent constriction and pD_2 were calculated from each of the dose-response curves. The maximum percent constriction was approximately the same (range 50–60%) for all of the arteriolar levels in all rats. There were no statistically significant differences in maximum percent constriction between any of the diabetic groups and their age-matched controls for the arterioles of three branching orders studied. In addition, there was no difference in maximum response among any of the ND or STZ-D rats for any order of arteriole, i.e., the maximum response did not change with age or duration of diabetes.

The calculated pD_2s for all vessels are presented graphically in Fig. 4. The mean ± SD pD_2 is plotted as bar height and paired for the STZ-D and ND rats in each diabetes-duration group (2 wk, 4 wk, etc.). Statistical comparisons among the ND rats revealed that there were no differences in sensitivity as a function of animal age for any of the branching orders of arterioles, i.e., the arteriolar sensitivity to norepinephrine in the ND rats was independent of the age of the animal within the age range studied. However, for the STZ-D rats, there were significant differences in sensitivity as a function of age and diabetes duration. For the 1A arterioles, sensitivity was normal (compared with age-matched ND rats) in all cases (2, 4, 8, and 16 wk after induction of diabetes), although there appeared to be a trend (not quite statistically significant at the 0.05 level) toward an increase in sensitivity after 16 wk. (As mentioned above, data were not obtained for 1A arterioles at 32 wk.) The 2A arterioles were hypersensitive at 2, 4, 8, and 16 wk, but sensitivity returned to normal after 32 wk. The 3A arterioles exhibited hypersensitivity at 2, 4, and 8 wk and normal sensitivity at 16 and 32 wk.

DISCUSSION

The objectives of this study were to determine if chemically induced diabetes is associated with any changes in microvascular sensitivity to norepinephrine and to investigate how that sensitivity changes as diabetes progresses from the acute to the chronic stage. The results varied somewhat depending on the location of the arteriole within the microvascular bed. For the larger 1A arterioles, normal sensitivity was observed at all stages of diabetes, although there appeared to be a tendency (not statistically significant) toward hypersensitivity at the later stages. The intermediate (2A) and smaller (3A) arterioles were hypersensitive during the early stages, but sensitivity returned to normal as diabetes progressed to the chronic stage. Although changes in microvascular sensitivity were apparent in this study, other measured variables were not significantly altered during diabetes. Maximum percent constriction and resting diameter for arterioles of all branching orders and mean arterial pressure were unchanged during diabetes.

Several previous studies have investigated vascular responses to norepinephrine in diabetes. Most of these studies have involved isolated tissue from large vessels and have not considered the potential effects of duration of diabetes on the measured responses. The results reported in these studies have varied considerably, some reporting increased and some decreased vascular sensitivity associated with diabetes. The reason for these differences is not clear but could involve factors such as differences in technique, severity of the diabetic state, or duration of diabetes at the time of the study. Comparison of the results of previous studies to this study are difficult because of the very different vascular tissues studied. This study involved direct measurement of arteriolar responses to norepinephrine in an intact microvascular bed in vivo. Although changes in large vessels such as the aorta during diabetes could certainly be important, it seems that measurements within the microcircu-

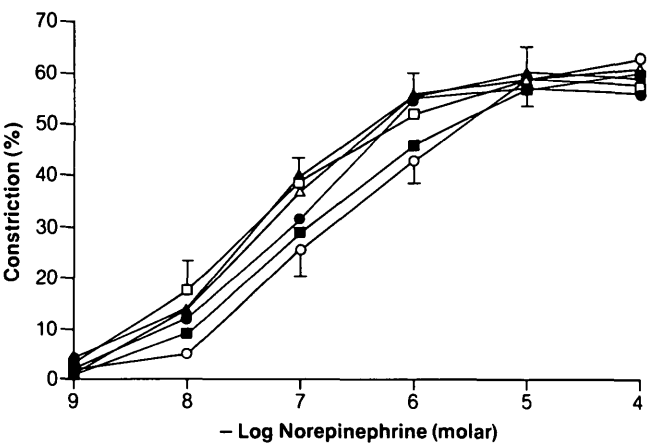

FIG. 2. Norepinephrine dose-response relationships for 2nd-branching-order arterioles. ■, Streptozocin-induced diabetic rats diabetic for 32 wk; other symbols as in Fig. 1.

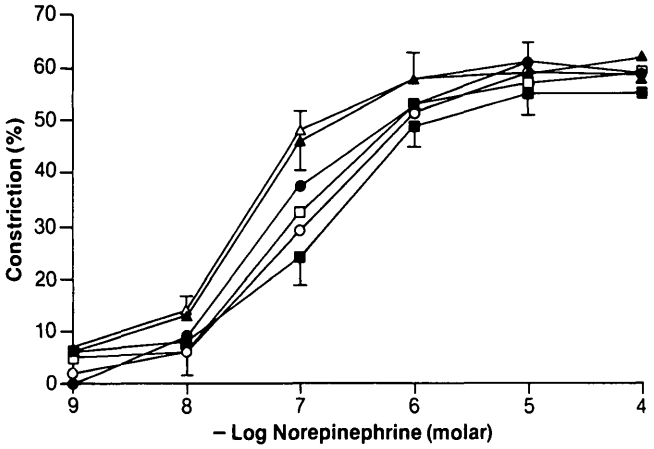

FIG. 3. Norepinephrine dose-response relationships for 3rd-branching-order arterioles. Symbols as in Fig. 2.

lation are more appropriate to interpretations of the potential causes of the major vascular complication associated with diabetes (microangiopathy). In addition, because the arterioles represent the major site of vascular resistance and are the vessels most actively involved in control of tissue perfusion, small changes in arteriolar sensitivity could have profound functional significance in the control of blood pressure and the regulation of tissue blood flow.

A few previous studies have measured microvascular responses to norepinephrine in diabetes. Joyner et al. (15) studied small arterioles in the hamster cheek pouch 1, 2, and 3 mo after induction of diabetes by STZ. They found no apparent changes in norepinephrine sensitivity for 1A, 2A, or 3A arterioles at any of the times studied. However, they observed a large vasodilation in response to norepinephrine in the cheek pouch in contrast to the vasoconstrictor action of norepinephrine in large doses that is reported in most other studies. The reason for this observed difference in response to norepinephrine is not clear, but it may be related to a difference in the distribution and density of α- and β-adrenergic receptors in the cheek pouch compared with other tissues such as the cremaster. The tissue-related difference in receptor types makes comparison between the results of the Joyner et al. study and this study difficult. If in fact the arterioles in the hamster cheek pouch respond to norepinephrine by constricting in the healthy animal but dilating in the diabetic animal, then this appears to be a phenomenon that has not been observed in other tissues but is certainly worthy of additional study. Rosenblum et al. (14) measured the responses of mesenteric arterioles to several vasoactive agents in healthy mice and mice with STZ-D for 7–8 wk. They reported that diabetic mice had significantly smaller responses than control mice to norepinephrine. However, this was true only when the responses were expressed as a percentage of the control diameter and then only for the highest dose of norepinephrine. Rosenblum et al. did not obtain complete dose-response curves and therefore could not measure overall sensitivity as determined by an ED_{50}.

In this study, there appeared to be a significant temporal component to the development of the observed microvascular changes; the smaller arterioles were hyperresponsive to norepinephrine during the first few weeks of diabetes, but the responsiveness returned to normal as diabetes progressed to the chronic stage. In the larger arterioles, results were different; the 1A arterioles responded normally throughout, although there appeared to be a tendency (not statistically significant) toward hyperresponsiveness after 16 wk. Unfortunately, because of the technical limitations mentioned previously, we were not able to determine if this apparent trend for the 1A vessels was more fully developed after 32 wk. These results are generally consistent with those recently reported by MacLeod and McNeill (10). They observed a supersensitivity to norepinephrine in aortas from diabetic rats initially, followed by a return to normal responsiveness at 100

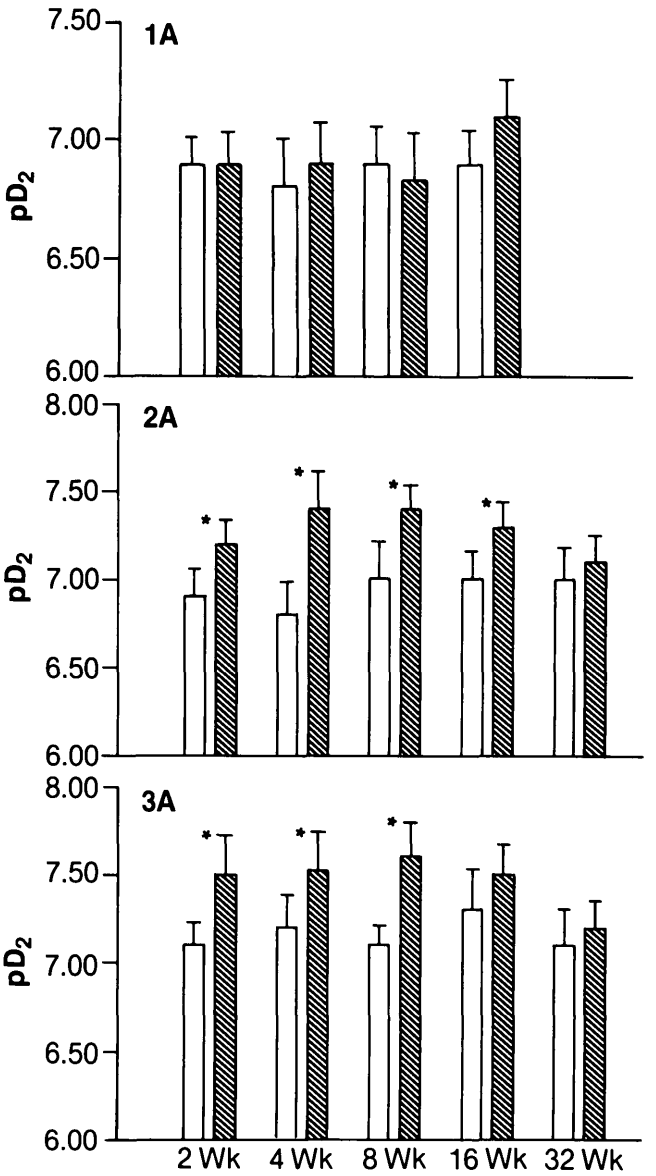

FIG. 4. Comparison of arteriolar sensitivity to norepinephrine in streptozocin-induced diabetic (*hatched bars*) vs. nondiabetic (*open bars*) rats. *Vertical bars*, calculated pD_2 ($-\log ED_{50}$) obtained from dose responses in Figs. 1–3. pD_2 *bars* are paired for each age and duration of diabetes (2 wk, 4 wk, etc.) and are shown for 1st-branching-order (1A), 2nd-branching-order (2A), and 3rd-branching-order (3A) arterioles. *Vertical lines*, SD. *$P < 0.05$.

days, but the vessels again became supersensitive at 180 days. The results observed in large vessels and the results of this study of the microcirculation suggest that there is indeed a significant temporal component to the development of the observed changes in reactivity and that the nature of the temporal changes may be rather complex. This finding could help explain some of the disparate results for diabetic vascular reactivity that have been reported in numerous studies.

In addition to the complex temporal development, there also appears to be a significant anatomic complexity to the observed microvascular reactivity changes observed in rats with chemically induced diabetes. This is evidenced on a macrovascular scale by the clear differences between arterial and venous responsiveness reported by MacLeod and McNeill (10) and on a microvascular scale by the significant differences in responses between large and small arterioles observed in this study. The underlying mechanisms for these anatomic differences in reactivity are not obvious, but the presence of specific differences in microvascular behavior, even at various levels of arteriolar branching within a given vascular bed, have been previously reported (23). In addition, there appears to be a distinct anatomic differentiation of α-receptor subtypes at different arteriolar levels in rat cremaster muscle (24). This would undoubtedly contribute to the complex pattern of responses to norepinephrine observed in healthy rats, and possible changes in the density and activity of the various receptor subtypes at different locations within the microcirculation could also explain some of the reactivity changes observed during diabetes. However, the details of how receptor (or other) changes could account for the observed reactivity changes remain to be elucidated by more detailed pharmacological studies.

One obvious question with regard to this study is, What relationship if any do reactivity changes observed in the microcirculation of an animal with chemically induced diabetes have to the development of microvascular disease in human diabetic patients? Although a clear extension of the animal results to human patients may not be possible, it does appear that some correlation may be present. Several studies of the effects of diabetes on vascular reactivity to various stimuli have been published (16,17,25–27). In all of these studies, indirect noninvasive assessments of microvascular function appeared to reveal functionally significant changes in vascular reactivity associated with the development of diabetes in human patients. For example, Christlieb et al. (4) demonstrated some years ago that diabetic patients with retinopathy exhibited enhanced vascular reactivity to angiotensin II and norepinephrine. Other investigators, such as Katz and McNeill (16), studied overall vascular reactivity in human diabetic patients by indirectly measuring the microvascular response to a change in physiological state. They found that cutaneous precapillary vessels in diabetic patients exhibited a depressed vasodilatory response to a standard exercise forcing. One possible explanation for this observation would be an increased responsiveness of the precapillary vessels to circulating or neurally released vasopressor agents, although other explanations are certainly possible. Some clinical investigators concluded several years ago that "abnormal vascular reactivity, whatever its underlying mechanism, is a newly recognized vascular complication of diabetes (28)."

In summary, the results obtained in this study involving direct observation of the intact microcirculation indicate that significant changes in microvascular reactivity to norepinephrine are associated with the development of STZ-D. Larger (1A) arterioles were normally responsive at all stages of diabetes. In contrast, smaller (2A and 3A) arterioles were hyperresponsive initially after diabetes induction but became normally responsive as diabetes progressed. These results support the concept that there are important functional alterations in the behavior of the microcirculation associated with the development of diabetes and that these functional changes are anatomically specific and temporally dependent.

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