The Effect of Insulin Treatment on Changes in Vascular Reactivity in Chronic, Experimental Diabetes

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SUMMARY

The influence of insulin treatment on the reactivity of aortae and mesenteric arteries from rats with chronic streptozocin (STZ)-induced diabetes was examined. Ninety days after the onset of diabetes, the responsiveness (developed tension [g]/cross-sectional area of tissue [mm^2]) but not the sensitivity (pD_2 value) of both aortae and mesenteric arteries from untreated rats to norepinephrine (NE) was significantly increased compared with age-matched, nondiabetic controls. However, responses of K^+-depolarized preparations from untreated diabetic rats to increasing extracellular Ca^{2+} were unchanged. Treatment of diabetic animals with daily injections of insulin for 90 days, starting 3 days after STZ treatment, normalized blood glucose levels and body weights and completely prevented the increases in responsiveness of aortae and mesenteric arteries to NE. No significant differences in systolic blood pressures, measured at weekly intervals, could be detected between nondiabetic, untreated diabetic, and insulin-treated diabetic rats. Insulin treatment of diabetic animals for 30 days, begun 90 days after the onset of diabetes, also normalized blood glucose levels and completely reversed the increases in the responsiveness of aortae and mesenteric arteries to NE. These results indicate that selective increases in the reactivity of aortae and mesenteric arteries to NE occur in diabetic rats before the development of hypertension. The ability of chronic insulin treatment to restore vascular responsiveness to NE to control levels suggests that the increased reactivity is a consequence of the diabetic state, and may predispose animals to the subsequent development of hypertension. DIABETES 1985; 34:1160–67.

The cardiovascular problems faced by patients with diabetes mellitus have been well documented. In addition to suffering from an increased incidence of cardiovascular diseases, such as hypertension and coronary heart disease,[1,2] diabetic patients are also susceptible to relatively specific forms of large and small blood vessel disease, known respectively as macro- and microangiopathies.[3] The reasons for the structural and functional deterioration of the cardiovascular system in diabetes are not yet completely understood, but the suggestion has been made that some of the manifestations of cardiovascular disease in diabetes arise from alterations in the reactivity of blood vessels to neurotransmitters and hormones.[4,5]

Vascular reactivity has been quite extensively studied in isolated preparations from animals with chemically induced diabetes, but the results of these studies appear to vary depending on the species and sex of the animal model used, the nature of the diabetogenic agent (either alloxan or streptozocin), the vascular preparation tested, and the duration of the diabetic state. We recently reported that aortae but not portal veins from female streptozocin (STZ)-treated rats exhibited a selective increase in sensitivity (pD_2 value) to norepinephrine (NE) after 100 days of diabetes, while after 360 days of diabetes, aortae were supersensitive and exhibited a greater maximum response to NE, 5-hydroxytryptamine, and KCl.[6] Aortae from male rats treated with STZ were reported to show a selective increase in maximum response to NE after 4–5 and 8–10 wk of diabetes,[7,8] while after 42–43 wk of diabetes, both the sensitivity and the maximum response of mesenteric arteries from STZ-treated animals to NE were increased.[9] Diabetic animals in the latter study were also found to be hypertensive compared with their age-matched controls.[9]

It is not clear whether the changes in reactivity of aortae and mesenteric arteries from diabetic animals occur at similar times after the onset of diabetes, or whether these changes in the reactivity of either vessel occur in conjunction with the development of overt hypertension. In addition, the influence of insulin treatment on the increases in vascular reactivity in chronic diabetic rats is unknown. However, if the increased

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TABLE 1
Some characteristics of untreated and insulin-treated rats* after 90 days of diabetes, and of age-matched, nondiabetic control rats

	N	Body wt (g)	Blood glucose (mg/dl)	Cross-sectional areas (mm^2)	
				Aortae	Mesenteric arteries
Nondiabetic controls	16	429 ± 9†	76.6 ± 4.7	1.17 ± 0.04	0.45 ± 0.02
Untreated diabetics	14	334 ± 10‡	605.4 ± 18.9‡	1.11 ± 0.04	0.38 ± 0.03‡
Insulin-treated diabetics	16	446 ± 11	107.0 ± 31.2	1.19 ± 0.03	0.46 ± 0.03

*Rats were given daily insulin injections from 3 days after induction of diabetes until the day of killing.
†Mean ± SEM.
‡$P < 0.05$ compared with nondiabetic and insulin-treated diabetic rats.

reactivity is a consequence of the diabetic state, then treatment of diabetic animals with insulin might be expected to prevent or reverse the severity of these changes. In the present investigation, the influence of diabetes of 90-day duration on the reactivity of aortae and mesenteric arteries and the systolic blood pressure of male rats was determined. The influence of insulin treatment begun just after the induction of diabetes (prevention study) on blood pressure and vascular reactivity was also measured, and the effect of insulin treatment on established changes in vascular reactivity (reversal study) was determined.

MATERIALS AND METHODS

ANIMALS

Male Wistar rats weighing 190–215 g were used in the present investigation. Animals were lightly anesthetized with ether, and either STZ (65 mg/kg) or the citrate buffer vehicle (pH 4.5) were administered by injection into the lateral tail vein. STZ- and vehicle-treated animals were housed separately, given free access to food and water, and were monitored for the development of glycosuria using Lilly Testape.

INSULIN TREATMENT PROTOCOLS

Prevention study. Three days after injection of STZ or vehicle, STZ-treated animals that had become glycosuric were divided randomly into two groups. One group received daily subcutaneous (s.c.) injections of 0.9 U insulin/100 g body wt. This dose of insulin had been previously established to maintain blood glucose levels of STZ-treated rats within the range of control animals.[10] The other group of diabetic animals and all nondiabetic controls received daily s.c. injections of saline. Systolic blood pressures were monitored on a weekly basis in a subgroup of control, untreated diabetic, and insulin-treated diabetic animals selected at random. Blood pressures were measured indirectly, using the tail-cuff method, in conscious, restrained animals after slight warming. Ninety days after STZ injection, animals were weighed and then killed by stunning followed by decapitation. Blood was collected for glucose assay and the blood vessels were removed and prepared as described below.

Reversal study. After STZ injection, rats remained untreated for 90 days. The diabetic animals were then divided randomly into two groups, one of which was treated for 30 days with insulin as described above. The second diabetic group and the nondiabetic controls were again injected with saline. One hundred and twenty days after STZ treatment, animals were weighed and killed.

PREPARATION OF BLOOD VESSELS AND EXPERIMENTAL PROTOCOL

The thoracic aorta and the superior mesenteric artery were excised from each animal and placed in Kreb's solution of composition (mM): NaCl (113), KCl (4.7), $CaCl_2$ (2.5), KH_2PO_4 (1.2), $MgSO_4$ (1.2), $NaHCO_3$ (25), and dextrose (11.5). The vessels were gently cleaned of fat and adventitia, and a 3-mm ring of mesenteric artery or a 5-mm ring of aorta was

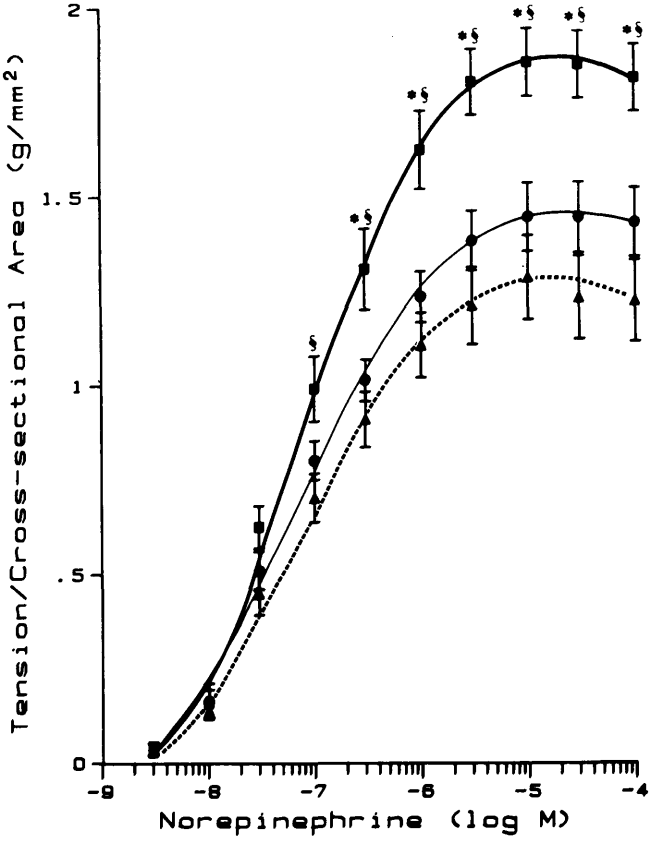

FIGURE 1. Norepinephrine dose-response curves in aortae from nondiabetic rats (●——●), untreated rats after 90 days of diabetes (■——■), and 90-day diabetic rats treated with insulin from 3 days after STZ injection until the day of killing (▲——▲). Each point represents the mean ± SEM of 7–9 observations. *$P < 0.05$ compared with nondiabetic; §$P < 0.05$ compared with insulin-treated diabetic.

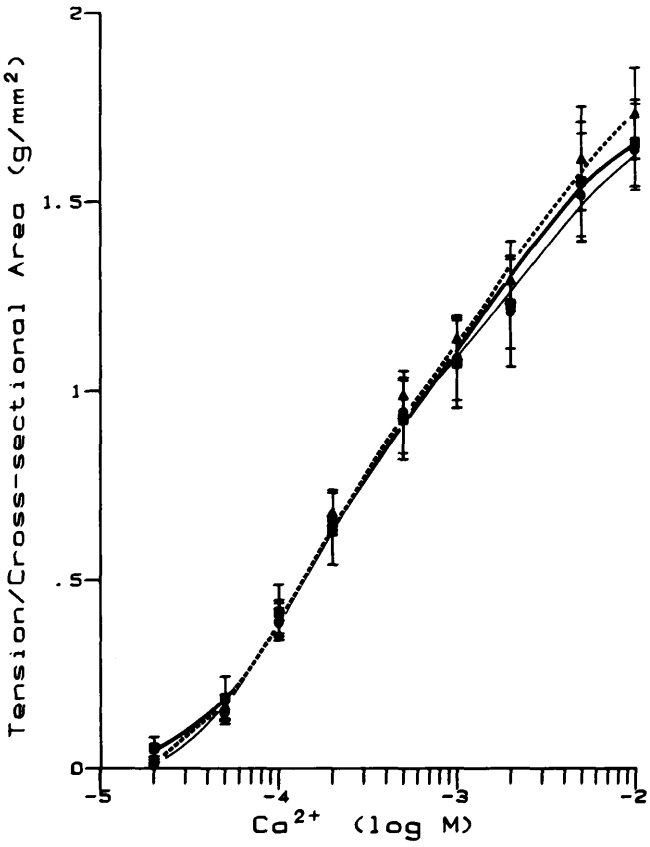

FIGURE 2. Dose-response curves to Ca^{2+} in K^+-depolarized aortae from nondiabetic rats (●——●), untreated rats after 90 days of diabetes (■——■), and 90-day diabetic rats treated with insulin from 3 days after STZ injection until the day of killing (▲——▲). Each point represents the mean ± SEM of 6–7 observations.

suspended between the bases of two triangular-shaped wires.[11] One wire was attached to a fixed support in a 20-ml isolated tissue bath containing Kreb's solution, maintained at 37°C, and oxygenated with 95% O_2:5% CO_2. The other wire was attached by cotton thread to a Grass FT-03 force displacement transducer. Responses were recorded on a Beckman Model 611 dynograph. In preliminary experiments, resting tension versus developed tension (in response to 10^{-7} M noradrenaline) curves were obtained in aortae and mesenteric arteries from 90-day diabetic and control animals. These curves were not significantly different in blood vessels from diabetic and nondiabetic animals, and the optimal resting tensions were determined to be 1.0 g in mesenteric artery and 2.0 g in aorta. Therefore, blood vessels were placed under the appropriate resting tension and were allowed to equilibrate for 90 min, with several changes of bathing solution, before beginning the dose-response curves.

Cumulative dose-response curves to NE were obtained in untreated tissues. Cumulative dose-response curves to $CaCl_2$ were obtained in tissues incubated in a high-K^+ Kreb's solution containing zero Ca^{2+}, 40 mM KCl (substituted for an equimolar amount of NaCl), and 10^{-7} M phentolamine (to block the effects of any neurally released NE). At the completion of each experiment, tissues were lightly blotted, measured, and weighed. The cross-sectional area of each preparation was calculated using the following formula: cross-sectional area (mm^2) = weight (mg)/length (mm) × density (mg/mm^3). The density of the preparations was assumed to be 1.05 mg/mm^3. The responsiveness of each preparation to NE and $CaCl_2$ was calculated as the increase in tension (g) in response to each concentration of agonist/cross-sectional area of tissue. The threshold dose of each agonist was taken to be the lowest concentration of agonist that first produced a measurable response. Agonist pD_2 values ($-\log ED_{50}$) were also calculated[12] and taken as a measure of the sensitivity of the tissues to each agonist.

Serum glucose levels were measured using the Ames blood analyzer glucose reagent kit.

Results were compared for significant differences using one-way ANOVA followed by Neuman-Keul's test. Results were considered significantly different if $P < 0.05$.

Drugs used in the present study were streptozocin (STZ), 1-norepinephrine hydrochloride (both from Sigma, St. Louis, Missouri), protamine zinc insulin (Iletin, Eli Lilly and Company, Indianapolis, Indiana), and phentolamine methanesulfonate (Ciba, Mississauga, Ontario, Canada).

RESULTS

Vascular reactivity in untreated and insulin-treated rats after 90 days of diabetes: prevention study. Ninety days after the onset of diabetes, untreated diabetic animals weighed significantly less than nondiabetic controls (Table

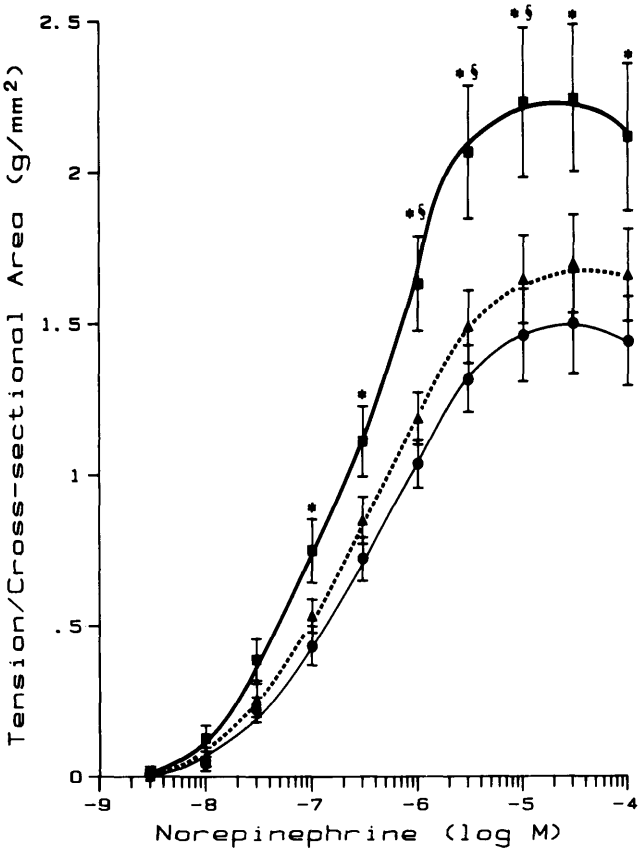

FIGURE 3. Norepinephrine dose-response curves in mesenteric arteries from nondiabetic rats (●——●), untreated rats after 90 days of diabetes (■——■), and 90-day diabetic rats treated with insulin from 3 days after STZ injection until the day of killing (▲——▲). Each point represents the mean of 8–9 observations. *P < 0.05 compared with nondiabetic; §P < 0.05 compared with insulin-treated diabetic.

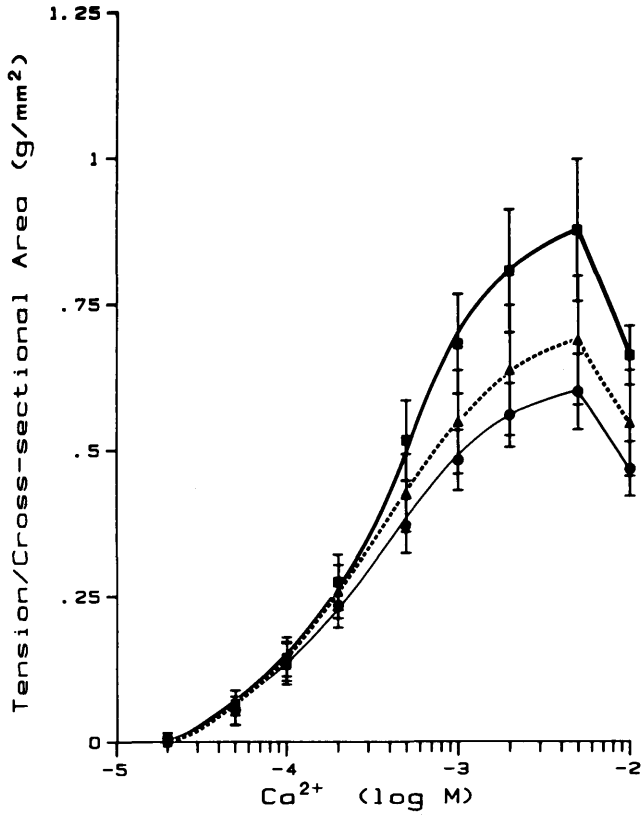

FIGURE 4. Dose-response curves to Ca^{2+} in K^+-depolarized mesenteric arteries from nondiabetic rats (●——●), untreated rats after 90 days of diabetes (■——■), and 90-day diabetic rats treated with insulin from 3 days after STZ injection until the day of killing (▲----▲). Each point represents the mean ± SEM of 6–7 observations.

1). In contrast, the weights of diabetic animals treated with insulin from the third day after STZ injection were very similar to those of control animals. Treatment of diabetic animals with insulin also tended to normalize serum glucose levels, which were much higher than control in untreated diabetic animals, but not significantly different from control in diabetic animals treated with insulin (Table 1). Two of the insulin-treated diabetic rats exhibited hyperglycemia at the time of killing as indicated by glucose levels >300 mg/dl. However, since the body weights of these animals fell within the range of the other insulin-treated diabetic rats, data from these animals were included with those of the insulin-treated diabetic group.

No significant differences in systolic blood pressures could be detected between the three groups of animals at any time after the induction of diabetes. Blood pressures after 3 mo of diabetes were 139 ± 7 (mean ± SEM, N = 4) in nondiabetic rats, 146 ± 7 (N = 6) in untreated diabetic animals, and 133 ± 6 (N = 5) in insulin-treated diabetic rats.

Blood vessels from diabetic animals were slightly smaller in cross-sectional area than those from nondiabetic animals (Table 1). However, treatment of diabetic animals with insulin prevented this decrease in size. Insulin treatment also prevented the change in the reactivity of diabetic aortae to NE. Concentrations of NE greater than 3×10^{-8} M produced much greater increases in tension in aortae from diabetic animals than in aortae from nondiabetic controls (Figure 1), although the threshold dose (3×10^{-9} M) and the NE pD_2 values (7.10 ± 0.08 [mean ± SEM] in controls and 7.08 ± 0.09 in diabetics) were similar in both preparations. In contrast, responses of aortae from insulin-treated diabetic animals to NE were very similar to those of aortae from nondiabetic rats (Figure 1). Despite the large differences in responsiveness of aortae from diabetic and nondiabetic rats to NE, responses of aortae from these two groups of animals, as well as from insulin-treated diabetic rats, to Ca^{2+} were almost superimposable (Figure 2). There were no significant differences in threshold dose, sensitivity, or responsiveness to Ca^{2+} between K^+-depolarized preparations from nondiabetic, untreated diabetic, and insulin-treated diabetic rats.

Responses of mesenteric arteries to NE are shown in Figure 3. As in aortae, concentrations of NE greater than 3×10^{-8} M produced much greater increases in tension in arteries from diabetic than nondiabetic rats, while insulin treatment prevented this increase in responsiveness. No change in the sensitivities of arteries from either untreated or insulin-treated diabetic rats compared with control was detected. NE pD_2 values were 6.53 ± 0.13 in control arteries, 6.60 ± 0.12 in arteries from untreated diabetic rats, and 6.54 ± 0.07 in arteries from insulin-treated diabetic rats. Mesenteric arteries from diabetic rats also responded to Ca^{2+} with greater increases in tension than arteries from nondiabetic animals, but in this case the differences in responsiveness were not significant, even at the maximum response (Figure 4). Re-

TABLE 2
Some characteristics of untreated and insulin-treated rats* after 120 days of diabetes, and of age-matched, nondiabetic control rats

	N	Body wt (g)	Blood glucose (mg/dl)	Cross-sectional areas (mm^2)	
				Aortae	Mesenteric arteries
Nondiabetic controls	14	413 ± 13†	85.5 ± 3.8	1.05 ± 0.02	0.43 ± 0.02
Untreated diabetics	12	257 ± 10‡,§	479.1 ± 30.5‡,§	0.90 ± 0.03‡,§	0.37 ± 0.02‡
Insulin-treated diabetics	11	307 ± 12‡	83.4 ± 33.2	1.01 ± 0.04	0.40 ± 0.04

*Rats were given daily injections of insulin for 30 days before time of killing.
†Mean ± SEM.
‡$P < 0.05$ compared with nondiabetic controls.
§$P < 0.05$ compared with insulin-treated diabetics.

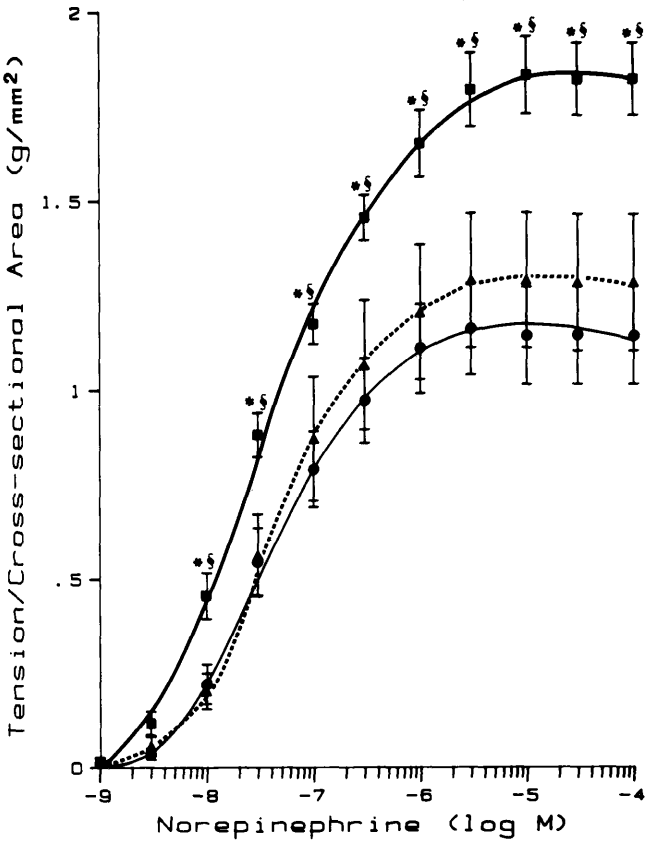

FIGURE 5. Norepinephrine dose-response curves in aortae from nondiabetic rats (●——●), untreated rats after 120 days of diabetes (■——■), and 120-day diabetic rats treated with insulin for 30 days before time of killing (▲----▲). Each point represents the mean ± SEM of 6–8 observations. *P < 0.05 compared with nondiabetic; §P < 0.05 compared with insulin-treated diabetic.

sponses of mesenteric arteries from insulin-treated diabetic animals to Ca^{2+} were intermediate between those of arteries from nondiabetic and insulin-treated diabetic animals (Figure 4). The sensitivities of arteries from the three groups of animals to Ca^{2+} did not differ from each other; the Ca^{2+} pD_2 values were 3.55 ± 0.06 in control arteries, and 3.50 ± 0.06 and 3.51 ± 0.04 in arteries from untreated and insulin-treated diabetic rats, respectively.

Vascular reactivity in untreated and insulin-treated rats after 120 days of diabetes: reversal study. One hundred and twenty days after the onset of diabetes, untreated diabetic rats weighed much less than nondiabetic animals (Table 2). Thirty days of insulin treatment resulted in some weight gain compared with untreated diabetic animals, but insulin-treated diabetic rats still weighed significantly less than nondiabetic rats (Table 2). Serum glucose levels, which were significantly elevated in untreated diabetic rats, were restored to near-control levels by treatment of diabetic animals with insulin (Table 2).

The cross-sectional areas of aortae and mesenteric arteries from untreated diabetic rats were considerably smaller than those of nondiabetic rats, while insulin treatment of diabetic animals partially reversed the decrease in vessel size (Table 2). As was found after 90 days of diabetes, the threshold dose of NE did not appear to differ from control in aortae from untreated diabetic rats (Figure 5). However, concentrations of NE greater than 3×10^{-9} M produced much greater increases in tension in aortae from diabetic than from control animals (Figure 5), although the NE pD_2 value in aortae from diabetic rats to NE (7.42 ± 0.10) was unchanged compared with control (7.37 ± 0.10). Insulin treatment for 30 days almost completely reversed the increased responsiveness of aortae from diabetic rats to NE (Figure 5) without affecting the pD_2 value of this agonist (7.28 ± 0.07). Responses of K^+-depolarized aortae from untreated diabetic rats and nondiabetic animals to Ca^{2+} did not differ significantly (Figure 6). However, responses of K^+-depolarized aortae from insulin-treated diabetic rats were depressed compared with those of the other two groups of aortae (Figure 6). While the maximum response of aortae from insulin-treated diabetic rats to Ca^{2+} was not affected, the Ca^{2+} dose-response curve was shifted to the right. The Ca^{2+} pD_2 value in aortae from insulin-treated diabetic rats was 3.45 ± 0.08, which was significantly less than the value in aortae from untreated diabetic rats (3.74 ± 0.09), although not significantly different than the value in aortae from nondiabetic controls (3.63 ± 0.03).

Responses of mesenteric arteries from untreated rats after 120 days of diabetes to suprathreshold concentrations of NE were much greater than those of arteries from nondiabetic animals (Figure 7). Insulin treatment again reversed the increased responsiveness of arteries from diabetic animals to

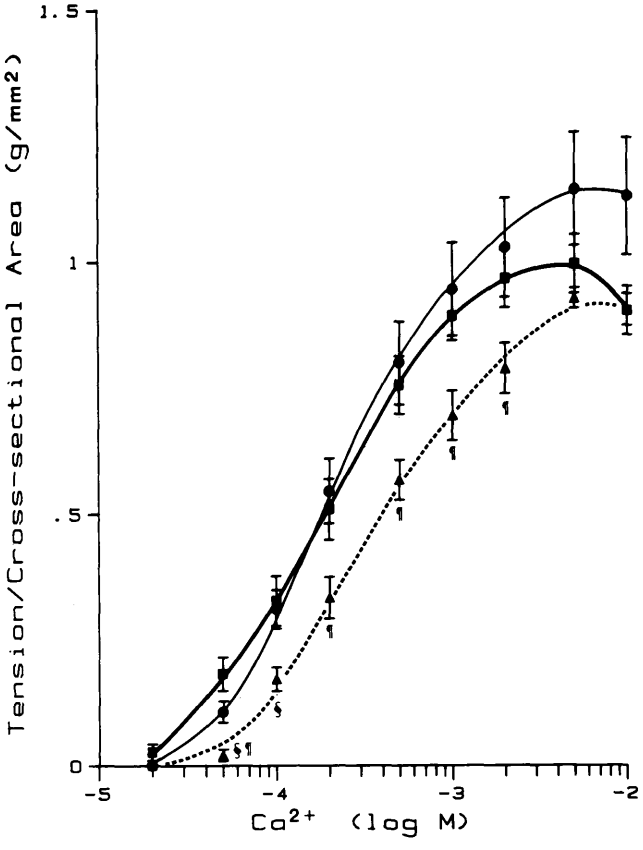

FIGURE 6. Dose-response curves to Ca^{2+} in K^+-depolarized aortae from nondiabetic rats (●——●), untreated rats after 120 days of diabetes (■——■), and 120-day diabetic rats treated with insulin for 30 days before time of killing (▲----▲). Each point represents the mean ± SEM of 4–6 observations. ¶P < 0.05 compared with nondiabetic; §P < 0.05 compared with untreated diabetic.

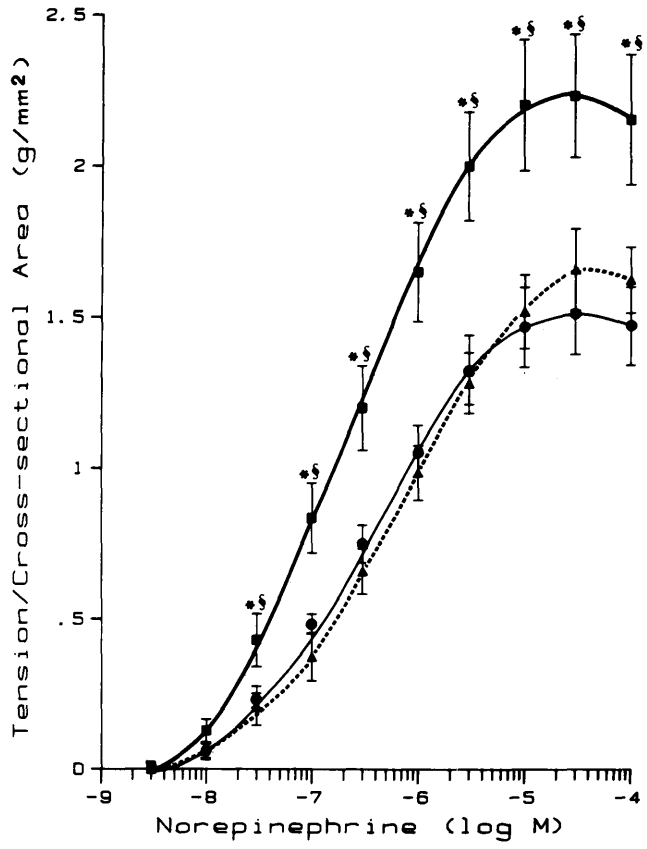

FIGURE 7. Norepinephrine dose-response curves in mesenteric arteries from nondiabetic rats (●——●), untreated rats after 120 days of diabetes (■——■), and 120-day diabetic rats treated with insulin for 30 days before time of killing (▲----▲). Each point represents the mean ± SEM of 6–8 observations. *P < 0.05 compared with nondiabetic; §P < 0.05 compared with insulin-treated diabetic.

this agonist (Figure 7). Insulin treatment also slightly decreased the sensitivity of mesenteric arteries from diabetic rats to NE. The NE pD_2 value in this preparation was 6.28 ± 0.11, which was significantly less than that of arteries from untreated diabetic rats (6.64 ± 0.08), but not significantly less than that of arteries from nondiabetic rats (6.54 ± 0.09). Responses of K^+-depolarized mesenteric arteries to Ca^{2+} are shown in Figure 8. Arteries from diabetic rats exhibited greater increases in tension in response to Ca^{2+} than arteries from nondiabetic animals, although the differences in responses between the two groups were not significant at any Ca^{2+} concentration. Insulin treatment reversed the increase in response to Ca^{2+} to slightly less than control levels. The Ca^{2+} pD_2 values of K^+-depolarized arteries from the three groups of animals did not vary significantly, being 3.56 ± 0.08 in nondiabetic controls, 3.47 ± 0.03 in untreated diabetics, and 3.35 ± 0.13 in insulin-treated diabetics.

DISCUSSION

The present investigation confirms previous reports that aortae from male rats with chronic STZ-induced diabetes are more responsive to the contractile effects of NE than are aortae from age-matched, nondiabetic animals.[7,8] This is in contrast to our previous findings of an increase in the sensitivity with no change in maximum responses of aortae from female rats with STZ-induced diabetes of the same duration,[6,13,14] which suggests that there are sex-related differences in the influence of diabetes on vascular reactivity. The increases in tension in response to NE in aortae after 90 and 120 days of diabetes found in the present investigation were not accompanied by changes in the reactivity of K^+-depolarized preparations to increased extracellular Ca^{2+}. Therefore, the increased responsiveness to NE probably does not result from a generalized increase in the contractility of the aortic smooth muscle, but rather reflects some selective change in the events leading to the NE response.

Responses of mesenteric arteries from untreated animals after 90 and 120 days of diabetes to NE were also increased compared with those of arteries from nondiabetic animals. However, this increase may be at least partially nonselective, since responses of K^+-depolarized mesenteric arteries from untreated diabetic rats to Ca^{2+} were also somewhat increased, although not significantly. It is possible that responsiveness of mesenteric arteries from diabetic rats to agonists may be enhanced by two different mechanisms, one involving a selective change in the events leading to the NE response, and the other a more generalized increase in the reactivity of the smooth muscle to agonists.

The mechanisms involved in producing the changes in reactivity of aortae and mesenteric arteries from diabetic rats to NE have not yet been elucidated. NE is a nonselective, α-adrenoceptor agonist that has been demonstrated to activate

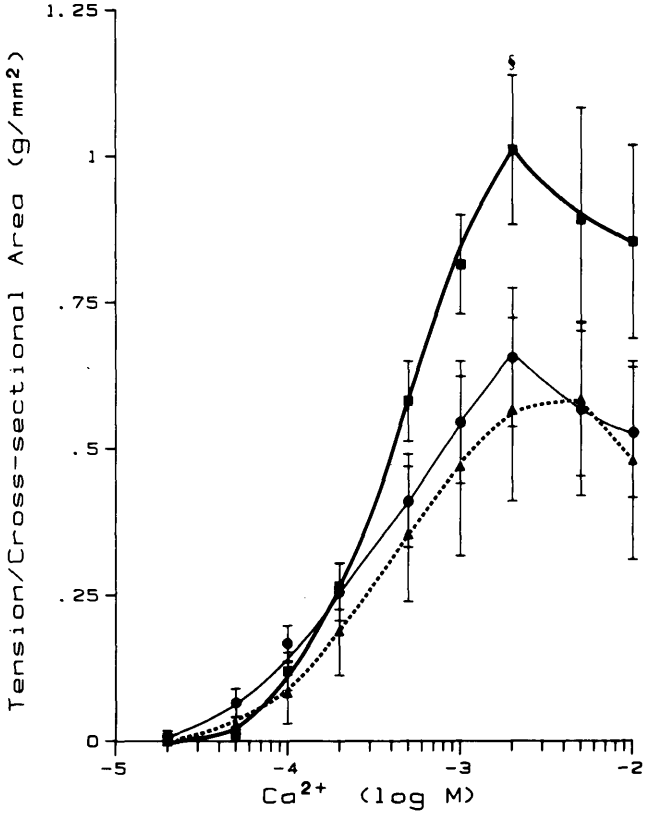

FIGURE 8. Dose-response curves to Ca^{2+} in K^+-depolarized mesenteric arteries from nondiabetic rats (●——●), untreated rats after 120 days of diabetes (■——■), and 120-day diabetic rats treated with insulin for 30 days before time of killing (▲----▲). Each point represents the mean ± SEM of 4–6 observations. §P < 0.05 compared with insulin-treated diabetic.

both α_1 and α_2 receptors. The exact nature of the α-adrenoceptors present in both rat aortae and mesenteric arteries, whether α_1, α_2, or both, is in some dispute.[15–19] However, in both arteries NE is believed to increase cytoplasmic Ca^{2+} levels by both increasing the influx of extracellular Ca^{2+} through receptor-operated channels, and by causing the release of intracellular Ca^{2+}.[16,20,21] Since the increases in tension of blood vessels from diabetic rats in response to NE found in the present investigation occurred in the absence of any changes in sensitivity (pD_2 value) to this agonist, it is unlikely that a change in either the density or the affinity of the α-receptors themselves occurred in blood vessels from diabetic rats. A more likely possibility is that there is an alteration in the coupling of the α-receptors to the mobilization of Ca^{2+}. Recently, Scarborough and Carrier[8,22] reported that the increased responsiveness of aortae from diabetic rats to NE was mediated by an increase in the activity of α_2-adrenoceptors, and resulted from an increase in the influx of extracellular Ca^{2+}. Whether such an explanation can also account for the increased responsiveness of mesenteric arteries from untreated diabetic rats to NE found in the present investigation is unclear. If Ca^{2+} influx is increased in aortae from diabetic rats, it is apparently only that associated with receptor-operated Ca^{2+} channels, and not that associated with potential-sensitive Ca^{2+} channels, since responses of K^+-depolarized preparations to increasing extracellular Ca^{2+} were not altered.

It is apparent from the results of the present investigation that treatment of diabetic animals with insulin both prevented the onset of the increased responsiveness of aortae and mesenteric arteries to NE, and reversed the increased responsiveness once it had occurred. These results imply that the increased responsiveness of isolated arterial preparations from STZ-treated animals is the result of the diabetic state, and not due to direct toxic effect of the diabetogenic agent. Studies in human patients with diabetes mellitus indicate that vascular complications, such as hypertension and microangiopathy, remain and even progress despite treatment of patients with insulin,[2,23] yet in the present investigation the reactivity of arteries from insulin-treated diabetic rats was essentially identical to that of control rats. It is possible that this was due to the relatively short duration of the diabetic state examined, and that had the diabetes been allowed to progress for a longer period of time it would have been more difficult to prevent or reverse the observed changes in vascular reactivity. This possibility is supported by the recent observation that diabetic microangiopathy, manifested as an increase in capillary basement membrane thickness, is completely reversible if drug treatment is begun very early in the course of the disease.[24]

The factor(s) responsible for producing the increased vascular reactivity observed in arteries from diabetic rats is unknown. In addition to the hyperglycemia and reduced insulin levels noted in the present investigation, STZ-induced diabetes of 90-day duration is accompanied by elevated serum lipid levels (J. H. McNeill, personal communication) and decreases in thyroid hormone levels.[10] The presence of hyperglycemia alone does not appear to be responsible for the increased vascular responsiveness, since responses of blood vessels from two insulin-treated diabetic animals that were hyperglycemic at the time of killing were not significantly different from those of normoglycemic insulin-treated diabetic rats (data not shown). It is possible that the absence of insulin rather than the presence of hyperglycemia produces enhanced responses to NE. Acute insulin infusion into normoglycemic rats has been shown to inhibit the vasoconstrictor responses to NE in rat tail artery,[25] an action that has been postulated to result either from enhancement of the ability of tissues to take up NE, or a direct dilatory effect of insulin on blood vessels (reviewed in ref. 26). Neither of these possibilities is likely to be operative in the present situation, since enhanced vascular responses of insulin-treated diabetic animals to NE were detected in vitro in the absence of insulin. However, the ability of insulin to prevent or reverse the increase in vascular reactivity in diabetic rats could be the result of a chronic effect of insulin treatment, either directly on vascular smooth muscle or indirectly via its action on glucose metabolism. Other factors that could contribute to the increases in vascular reactivity to NE include decreases in thyroid hormone levels and malnutrition. The former possibility seems unlikely to result in the increased responsiveness to NE found in arteries from diabetic rats, since thyroidectomy has been reported to result in no change in the reactivity of either aortae[27] or mesenteric arteries[28] to NE. Although a possible contribution of malnutrition cannot be ruled out, caloric restriction has been reported to result in increases in the tension developed by isolated rat aortae in response to high K^+,[29] an observation that differs from the finding of the present investigation that responses of K^+-depolarized arteries from diabetic rats to Ca^{2+} were not changed.

Aortae from rats with STZ-induced diabetes have been reported to exhibit a nonselective depression of responses to both phenylephrine and KCl from 2 to 12 wk after the onset of diabetes.[30] The reason for the differences between the results of the previous study[30] and this one is not clear, but may be related to differences in the severity of the diabetic state. Insulin treatment of diabetic animals also prevented the depression of vascular reactivity found in aortae from diabetic rats.[30] It is interesting that no matter what the direction of the changes in responsiveness, insulin treatment of diabetic animals appears to prevent the onset of these changes.

In the reversal study, it was noted that aortae from diabetic animals treated with insulin for 1 mo were less sensitive to Ca^{2+} than were aortae from nondiabetic rats. The significance of this observation is not clear, since aortae from diabetic animals treated with insulin for 3 mo did not show a similar loss of sensitivity to Ca^{2+}. In addition, insulin treatment for 1 mo had no significant effect on the sensitivity of mesenteric arteries from diabetic rats to Ca^{2+}. However, there was a trend toward decreased sensitivity to both NE and Ca^{2+} in aortae and mesenteric arteries from diabetic animals treated with insulin for 1 mo. Again, it is not clear whether this represents a direct depressant effect of insulin on vascular smooth muscle or is the result of an extension of the normal metabolic actions of insulin.

No significant effect of diabetes of 90-day duration on blood pressure could be detected in the present investigation. Others have reported that systolic blood pressures of STZ-diabetic rats are either unchanged[31] or increased,[32–34] although the time of onset and magnitude of the increase

varied between studies. It is possible that blood pressures would have eventually increased in the diabetic animals used in this study had the animals been followed for longer periods. The increase in responsiveness of arteries from diabetic animals to NE occurs some time between 40 and 90 days after STZ injection (unpublished observations). If this contributes to the eventual development of hypertension, as has been postulated,[9] then the development of hypertension might be expected to be delayed beyond the development of enhanced responsiveness to NE.

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