Comparison of effects of diabetes mellitus on an EDHF-dependent and an EDHF-independent artery

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The endothelium is an important modulator of vascular smooth muscle tone and reactivity, and there is broad consensus that endothelial vasodilator dysfunction may contribute to the widespread vascular complications of diabetes mellitus. However, what is not clear is which vascular beds or which endothelial factor(s) may be implicated in this dilator dysfunction. For example, Kiff and colleagues (11) pointed out that, whereas the blood pressure response in intact conscious diabetic rats to infusion of endothelium-dependent and endothelium-independent dilators appears normal, the responses in different individual vascular beds vary. Such differences may reflect, at least in part, differences in the contribution of the various endothelium-dependent vasodilators in different vascular beds. For example, endothelium-dependent relaxation to acetylcholine (ACh) in the femoral artery is almost entirely dependent on nitric oxide (NO), whereas other endothelium-dependent vasodilators, especially endothelium-derived hyperpolarizing factor (EDHF), clearly play an important role in the mesenteric artery (20). Furthermore, not all agents stimulate the release of the same complement of vasodilators from endothelial cells. ACh stimulates release of NO and evokes an EDHF response in mesenteric arteries (7), whereas bradykinin stimulates predominantly NO from that vascular bed (16). These heterogeneous physiological effects of diabetes on the microcirculation may help to explain the differential impact of diabetes on the function of different vascular beds, which characterizes the clinical complications of this condition. The importance of this heterogeneity may be in directing further examination of the mechanisms underlying the specific changes in vasodilator responses in various parts of the microcirculation.

In the rat mesenteric artery a clear component of ACh-induced relaxation is resistant to blockers of NO and prostacyclin synthesis, suggesting a prominent role for EDHF in this vessel. Whereas most studies of endothelial dysfunction in diabetes have focussed on NO, Taylor and colleagues (16) reported impairment of the NO- and prostacyclin-resistant relaxation in mesenteric arteries of diabetic rats. Furthermore, the hyperpolarization attributed to EDHF in this artery is reduced in diabetes (7). These observations point to impairment of EDHF during diabetes, and we have now tested this hypothesis further by comparing endothelium-dependent responses in the mesenteric artery with those in an EDHF-independent vessel, namely, the femoral artery, which appears to depend predominantly on NO for endothelium-dependent vasodilation. Fukao and colleagues (7) studied membrane potential in diabetic rat mesenteric arteries in the absence of depolarization and constriction, and the results suggest impairment of EDHF in this vascular
bed as a consequence of diabetes. In the present study we tested this hypothesis further by comparing the effects of diabetes on endothelium-dependent vasodilation in the femoral artery, which appears to depend solely on NO, with the responses in the mesenteric artery, in which both NO and EDHF are released from the endothelium. We recorded membrane potential simultaneously with tension in both arteries depolarized and constricted with phenylephrine to more fully elucidate the contribution by EDHF to relaxation. This is the first documentation of such simultaneous recordings in constricted arteries of diabetic rats. As an alternative approach in studying the impact of diabetes, we also compared, in an individual artery (mesenteric, see above), the hyperpolarizations and relaxations evoked by two different agonists, ACh and bradykinin, each of which releases a different suite of dilators from the endothelium.

**METHODS**

**Animals and tissues.** Diabetes mellitus was induced in 8-wk-old adult male Wistar rats by injecting 60 mg/kg streptozotocin (STZ), dissolved in 0.1 M citrate buffer, into the tail vein under halothane and N₂O anesthesia. Nondiabetic age-matched control rats were also anesthetized, and blood glucose levels were determined in all animals. The rats were obtained from Monash University Central Animal Services (Monash University Animal Ethics Committee approval number P540 PHY/1997/028). Each diabetic rat was housed together with its control rat, and all rats were given free access to food and water. After 8 wk the rats were anesthetized with chloroform, weighed, and killed by decapitation, and blood samples were obtained for glucose determination. The mesenteric beds and femoral arteries were removed and placed in Krebs solution containing (mM) 120.0 NaCl, 5.0 KCl, 25.0 NaHCO₃, 11.0 glucose, 1.0 KH₂PO₄, 1.2 MgSO₄, and 2.5 CaCl₂, bubbled with 5% CO₂-95% O₂.

Distal branches of the femoral artery and tertiary branches of the mesenteric arteries (both 200- to 300-μm outside diameter) were dissected free from adipose and connective tissue, and ring segments 1–2 mm in length were separately mounted on a small vessel Mulvany-style myograph (12) for measurement of isometric tension. The arteries were continuously superfused with Krebs solution (3 ml/min) at 35°C and were stretched in increments to a tension that was equivalent to a transmural pressure of 80–90 mmHg, the mean arterial blood pressure in rats. An initial test application of the α₁-adrenoceptor agonist phenylephrine (10 μM) and ACh (10 μM) confirmed the integrity of the smooth muscle and endothelium, respectively, and the preparations were then allowed to recover for 30 min.

**Contraction and endothelium-independent relaxation.** Cumulative concentration-contraction curves to phenylephrine were performed to assess the ability of the muscle to contract. The ability of the smooth muscle to relax independently of the endothelium was tested using cumulative concentration-contraction curves to phenylephrine. This was added to the superfusate and, after 30 min, a third concentration-relaxation curve to ACh was performed.

**Initial control experiments.** From the concentration-response curves to phenylephrine, the concentrations of that drug that yielded similar levels of submaximal contraction were determined. Phenylephrine (3 μM) produced 79 ± 3% and 81 ± 4% contraction in femoral arteries from control and diabetic rats, respectively, and 10 μM induced 78 ± 4% and 78 ± 3% contraction in mesenteric arteries from control and diabetic rats, respectively (n = 10 rats in all 4 groups).

Because each tissue was subjected to three concentration-response curves, control, plus L-NAME, and L-NAME plus indomethacin, a series of time-control experiments without L-NAME or indomethacin was run. Three concentration-relaxation curves, with 30-min intervening, were indistinguishable.

The concentration of L-NAME required to achieve a reproducible rightward shift in the concentration-relaxation curve in response to ACh was verified in mesenteric arteries from five control rats. The pD₂ for ACh-evoked relaxation was 7.16 ± 0.08 and the maximum response (Vₘₐₓ) was 11 ± 5% of the phenylephrine-induced contraction (n = 7) in the presence of 50 μM L-NAME. Increasing the concentration of L-NAME to 100 μM shifted the pD₂ to 7.02 ± 0.14 (P > 0.05) and Vₘₐₓ to 20 ± 7% (P = 0.36), which were not significantly different compared with the lower concentration of L-NAME. Increasing the concentration of L-NAME to 1 mM had no further effect.

Finally, in most previous studies, ACh has been applied cumulatively when stimulating endothelium-dependent relaxation. When recording membrane potential simultaneously with tension, we have routinely applied ACh discretely, each application lasting 1 or 2 min with a return of the tension to its prerelaxation level between each application (15). Using mesenteric arteries obtained from five control rats, we constructed concentration-relaxation curves to ACh applied both cumulatively and discretely to each tissue. Both curves were identical using the two methods.

**Histology.** Segments of femoral and mesenteric arteries from diabetic and control rats were dissected out, fixed in 10% phosphate-buffered formalin, mounted in paraffin blocks, and cut into 10-μm slices for staining with Masson’s trichrome. This stain distinguishes connective tissue from smooth muscle cells and therefore enabled verification of the muscular status of the arteries that were studied.
Drugs. Drugs used in this study were the following: STZ, phenylephrine hydrochloride, ACh hydrochloride, l-NAME, indomethacin, bradykinin acetate, apamin, and superoxide dismutase (S-2515 from bovine erythrocytes) (Sigma; St. Louis, MO); charybdoxin (Auspep, Melbourne, Australia), SNP (Ajax Chemicals; Auburn, New South Wales, Australia); and 1-ethyl-2-benzimidazolinone (EBIO, Tocris, Bristol, UK). Phenylephrine, ACh, l-NAME, and bradykinin were prepared as stock solutions in distilled water, indomethacin in Na2CO3 (0.1 M), and EBIO in dimethyl sulfoxide.

Statistical analysis. Relaxations were expressed as a percentage of the contraction induced by phenylephrine, and hyperpolarization was expressed as the change in membrane potential (mV). Concentration-response curves were calculated, and a sigmoid curve was fitted to data for each artery using the least-squares method (Prism, Graphpad Software). The concentration of agonist that was effective in producing a response that was 50% of the maximum response (EC50), the maximum response induced by ACh (Vmax), and the slope were determined for each curve. For each group of data the mean values for EC50, Vmax and slope ± SE for each concentration of agonist were calculated, and a curve representative of each group was produced. pD2 (−log EC50) values have been quoted throughout when comparing responses of arteries to ACh, bradykinin, phenylephrine, and SNP.

All values are quoted as means ± SE based on the number of rats (n) in each group. Observations in different groups were initially compared using two-way ANOVA. This was followed by pair-wise statistical comparisons before and after incubation in L-NAME and indomethacin, using unpaired Student’s t-tests. A probability (P) < 0.05 was considered statistically significant.

RESULTS

Blood glucose levels and animal weights. At the time of the experiment, all STZ-treated rats exhibited hyperglycemia with blood glucose concentrations (31.0 ± 1.5 mmol/l, n = 10) significantly higher than those of the age-matched nondiabetic control rats (5.8 ± 0.5 mmol/l, n = 9, P < 0.0001). The weights of the rats at the time of injection were similar (293 ± 9 g, n = 10, for the diabetic rats; 281 ± 9 g for the controls, n = 9, P = 0.37), but the final weight of the diabetic rats (309 ± 14 g, n = 10) was significantly lower than that of the control rats (501 ± 23 g, n = 9).

ACh-induced relaxation in mesenteric versus femoral arteries of control rats. In nondiabetic control rats, endothelium-dependent relaxation of the femoral arteries required higher concentrations of ACh (pD2, 6.52 ± 0.07, n = 5) than the mesenteric arteries (pD2, 7.62 ± 0.04, n = 9, P < 0.001). Whereas the highest concentrations of ACh completely relaxed the mesenteric artery (only 1 ± 3% of the phenylephrine-induced contraction remained, n = 9), the maximum tension remaining in the femoral artery was 43 ± 9% (n = 5, P = 0.01) (Fig. 1).

Effect of l-NAME and indomethacin on ACh-induced vasodilatation in arteries of control rats. l-NAME (50 μM) abolished endothelium-dependent relaxation to ACh in the femoral artery (Fig. 2). This contrasted with observations in the mesenteric artery in which almost complete relaxation persisted in the presence of l-NAME, although there was a significant rightward shift in the pD2 (P < 0.0001) (Table 1, Fig. 3B). The addition of indomethacin, in the continued presence of l-NAME, had no further impact on endothelium-dependent relaxation to ACh in the mesenteric artery (Table 1, Fig. 3B).

Diabetes and ACh-induced vasodilatation. In femoral arteries, there was no difference between the concentration-relaxation curves to ACh in tissues from diabetic rats compared with nondiabetic control tissues (Table 1, Fig. 2). Likewise, l-NAME abolished the relaxation response to ACh in tissues from diabetic rats as it had in controls (Fig. 2).

In mesenteric arteries in control solution, the concentration-relaxation curves to ACh were shifted to the right in arteries from diabetic rats compared with those from nondiabetic controls, but the Vmax was not different and almost complete relaxation of the phenylephrine-induced contraction was achieved in both groups (Table 1, Fig. 3A).

In the presence of l-NAME, the pD2 for ACh-induced relaxation in mesenteric arteries from diabetic rats was shifted significantly to the right (Table 1, Fig. 3B). The shift in pD2 in the diabetic arteries by l-NAME was 0.50 ± 0.19 (n = 10), which was not different from the shift of 0.42 ± 0.08 (n = 9) in controls (P = 0.10, Table 1). However, the reduction in Vmax by l-NAME was significantly lower than that of the control rats (501 ± 23 g, n = 9).
was significantly greater in the mesenteric arteries from the diabetic rats compared with controls (Table 1, Fig. 3B). L-NAME reduced $V_{\text{max}}$ by only 6 ± 5% in controls ($n = 9$) compared with 35 ± 10% in diabetics ($n = 10, P = 0.02$).

Incubation in the free radical scavenger superoxide dismutase (150 U/ml for 20 min before and during testing) failed to improve the concentration-relaxation curves in response to ACh ($n = 5$) in mesenteric arteries obtained from either control or diabetic rats (data not shown).

Indomethacin was without further effect on the concentration-relaxation curves (pD$_2$ or $V_{\text{max}}$) for ACh in mesenteric arteries from diabetic rats (Table 1, Fig. 3B). The maximum relaxation evoked by ACh in mesenteric arteries in the presence of L-NAME plus indomethacin was abolished by 5 min exposure to charybotoxin (30 nM) plus apamin (0.25 μM) ($n = 3$). The effect of these blockers was reversed 30 min after they had been washed out.

**Hyperpolarization evoked by ACh in mesenteric and femoral arteries.** The resting membrane potentials of the smooth muscle cells were not different in mesenteric arteries of diabetic rats ($-58 \pm 2$ mV, $n = 6$) compared with controls ($-59 \pm 2$ mV, $n = 6$). When the arteries were depolarized and constricted with phenylephrine, the membrane potentials were also similar (diabetic, $-34 \pm 1$ mV, $n = 6$; control, $-35 \pm 1$ mV, $n = 5$).

In the presence of phenylephrine, ACh evoked substantial concentration-dependent hyperpolarization of the smooth muscle cells in the mesenteric arteries of control rats, with a markedly smaller response in tissues from diabetic rats (Fig. 4). This was reflected in significant differences in the pD$_2$ values and $V_{\text{max}}$ for ACh-induced hyperpolarizations in mesenteric arteries of controls versus diabetic rats (Table 2, Fig. 5). A combination of L-NAME and indomethacin reduced the hyperpolarization in mesenteric arteries of diabetic rats but not in controls (Table 2, Fig. 5).

The ACh-evoked hyperpolarization in femoral arteries of control rats was small in control solution. There was no difference in the pD$_2$ values or $V_{\text{max}}$ for ACh-induced hyperpolarization between tissues from control and diabetic rats (Table 2). The response was completely abolished in L-NAME (Table 2, Fig. 5).

### Table 1. pD$_2$ and $V_{\text{max}}$ values for concentration-relaxation curves to acetylcholine and bradykinin in mesenteric and femoral arteries from nondiabetic and diabetic rats before and after incubation with L-NAME and L-NAME plus Indo

<table>
<thead>
<tr>
<th></th>
<th>Control Solution</th>
<th>L-NAME</th>
<th>L-NAME + Indo</th>
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<tr>
<td><strong>Acetylcholine</strong></td>
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<tr>
<td>Mesenteric artery</td>
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<tr>
<td>Control</td>
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<td>$7.20 \pm 0.06^\dagger$</td>
<td>$7.09 \pm 0.06^\dagger$</td>
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<tr>
<td>Diabetic</td>
<td>$7.02 \pm 0.11^*$</td>
<td>$6.52 \pm 0.16^*\dagger$</td>
<td>$6.42 \pm 0.11^*\dagger$</td>
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<td>Femoral artery</td>
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<tr>
<td>Control</td>
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<td>$7.50 \pm 0.77$</td>
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<td>$91 \pm 4$</td>
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<td><strong>Bradykinin</strong></td>
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<tr>
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<td>$7.50 \pm 0.16$</td>
<td>$35 \pm 5$</td>
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</tr>
<tr>
<td>Diabetic</td>
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<td>$34 \pm 7$</td>
<td>$100$</td>
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<tr>
<td><strong>Acetylcholine</strong></td>
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<td>Femoral artery</td>
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<tr>
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<tr>
<td>Diabetic</td>
<td>$6.44 \pm 0.12$</td>
<td>$42 \pm 7$</td>
<td>$100$</td>
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Values are means ± SE; $n$, number of rats. $V_{\text{max}}$, maximal response; L-NAME, N$^\text{N}$-nitro-L-arginine methyl ester (100 μM); Indo, indomethacin (1 μM). *$P < 0.05$, significant difference between values for arteries from diabetic rats compared with control rats. †$P < 0.05$, significant difference between values in the presence of L-NAME and Indo compared with control solution.
Bradykinin-induced responses and the effect of diabetes. In mesenteric arteries, there was no difference between the concentration-relaxation curves to bradykinin in tissues from diabetic rats versus controls (Table 1, Fig. 6). L-NAME markedly reduced the bradykinin-evoked relaxation in arteries from both diabetic rats and controls (Table 1, Fig. 6). Addition of indomethacin, in the presence of L-NAME, abolished all bradykinin-induced relaxation (Table 1, Fig. 6).

Bradykinin was equipotent in evoking hyperpolarization in the smooth muscle cells of mesenteric arteries of both control and diabetic rats, and the $V_{max}$ values were also similar in the two groups (Table 2). This hyperpolarization was abolished in the presence of L-NAME plus indomethacin (Table 2).

In femoral arteries, bradykinin failed to evoke relaxation or hyperpolarization in tissues from either control or diabetic rats (data not shown).

Effects of EBIO. A significant proportion of the hyperpolarization evoked in the mesenteric artery by ACh in this study persisted in the presence of L-NAME plus indomethacin and hence was attributed to EDHF. Because the EDHF response is blocked by the combination of charybdotoxin and apamin in a variety of vascular smooth muscles (4, 6, 19), it has been ascribed to the activation of intermediate- and small-conductance, $Ca^{2+}$-activated $K^+$ channels. The intermediate-
conductance channels can be activated by EBIO (10). In the present study, EBIO evoked concentration-dependent hyperpolarization and relaxation in tissues obtained from control rats, whereas the responses in tissues from diabetic rats were absent or significantly reduced (Fig. 7).

**Endothelium-independent constriction and relaxation of arterial smooth muscle in diabetic and control rats.** The ability of phenylephrine to evoke vasoconstriction was not different in the mesenteric arteries from diabetic (pD2, 5.60 ± 0.03, n = 6) compared with control rats (pD2, 5.52 ± 0.02, n = 8, P = 0.08). Thus the ability of the smooth muscle to contract was unaffected by diabetes.

Likewise, the ability of the smooth muscle to relax was preserved in arteries obtained from diabetic rats. In mesenteric arteries, the pD2 for SNP-induced relaxation was 7.45 ± 0.07 (n = 5) for control rats and 7.57 ± 0.07 (n = 5) in tissues from diabetic rats (P = 0.26). V_max values for these responses were 3 ± 4% and 2 ± 3% of the phenylephrine-induced contraction, respectively (P = 0.85). In femoral arteries, the relaxations evoked by 10 μM SNP were 64 ± 9% (n = 5) and 79 ± 8% (n = 4) in control and diabetic rats, respectively (P = 0.26).

**Histology.** The media of both femoral and mesenteric arterial walls stained red with Masson’s trichrome indicating their status as muscular arteries. In the control rats the muscle layer was 6.4 ± 0.2 smooth muscle cells thick in the femoral artery (n = 8) and 4.3 ± 0.3 cells thick in the mesenteric artery (n = 8). In the diabetic rats the average number of muscle cells in the wall was 6.3 ± 0.3 in the femoral artery (n = 4) and 4.0 ± 0.3 in the mesenteric artery (n = 7), not different from the controls. Whereas in the dissecting dish in the absence of pressure, the outside diameter of all the arteries studied was 200–300 μm.

**DISCUSSION**

This study demonstrates that the impact of STZ-induced diabetes on the vasodilator function and control of the resistance circulation in rats is heterogeneous. The importance of this heterogeneity is that it...
appears to reflect an impairment of the functional response to EDHF rather than NO. Evidence to support this conclusion includes the finding that L-NAME has a greater impact on ACh-evoked maximum relaxation and hyperpolarization in mesenteric arteries from diabetic rats than from controls, suggesting that NO bioavailability, at least in vitro, is not reduced in the arteries of diabetic rats. Furthermore, definitive evidence provided by simultaneous recordings of membrane potential and tension confirmed that EDHF-dependent hyperpolarization and the associated relaxation is markedly reduced in mesenteric arteries from diabetic rats compared with controls. Finally, relaxation that relies predominantly on the release of NO from the endothelium, i.e., ACh-induced relaxation in the femoral artery and bradykinin-induced relaxation in the mesenteric artery, is resistant to the effects of diabetes. This implicates EDHF rather than NO in the endothelium-dependent vasodilator dysfunction in this model of diabetes.

Most studies examining the impairment of endothelial vasodilator function associated with diabetes have focused on the role of NO, and conflicting conclusions have been reached (see Ref. 5). The greater reduction of both maximum relaxation and maximum hyperpolarization by L-NAME in mesenteric arteries of diabetic rats suggests a greater dependence on NO in diabetes. Thus our results support those that suggest NO production may be unchanged or even increased in mesenteric arteries of diabetic rats (see Refs. 5 and 9). An increase in the burden of destructive reactive oxygen species in diabetes (2, 17) in vivo would reduce the bioavailability of NO, which could stimulate an increase in NO production (see Ref. 5).

The prominence of L-NAME-resistant relaxation to ACh in the mesenteric artery provides strong evidence that endothelium-dependent vasodilators in addition to NO are important in these vessels (7, 18, 20). Our electrophysiological recordings confirm that EDHF plays an important role in the ACh-evoked, endothelium-dependent relaxation of mesenteric arteries, and that this pathway is compromised following 8 wk of diabetes. This conclusion supports the observations of Fukao and colleagues (7) who recorded membrane potential in rat mesenteric arteries pinned to the bottom of a recording bath and not exposed to constrictor. They showed that the amplitude and duration of the hyperpolarization produced by acetylcholine was significantly decreased in arteries of diabetic rats. Blockade of NO synthesis did not affect the hyperpolarizing response to ACh in either diabetic animals or controls in that study and, furthermore, in vitro application of superoxide dismutase, a scavenger of superoxide anions, was without effect on the ACh-induced hyperpolarization. In our study L-NAME reduced the hyperpolarization in the diabetic arteries. The 31-mV hyperpolarization attributed to EDHF dominated the membrane potential response in control arteries and probably masked the smaller NO-dependent hyperpolarization. In the diabetic arteries the EDHF response was markedly reduced, revealing the NO-dependent hyperpolarization, and this hyperpolarization was reduced by L-NAME. NO-dependent hyperpolarization is sensitive to the degree of stretch on the artery (14) and thus more likely to be detected in arteries mounted on a myograph than in arteries pinned to the base of a recording chamber. Nonetheless, our results confirm and extend Fukao et al.’s initial observations (7), lending support to the notion that the functional EDHF response is decreased in mesenteric arteries during diabetes in rats. Simultaneous measurement of membrane potential and tension in our study enabled us to unequivocally demonstrate that impaired hyperpolarization underpinned the reduced ACh-induced relaxation in these arteries.

Fig. 7. Hyperpolarization and relaxation evoked in the smooth muscle cells of mesenteric arteries from control and diabetic rats by 1 and 10 \( \mu M \) 1-ethyl-2-benzimidazolinone (EBIO). Values are means ± SE (n).
We have also tested the hypothesis further by recording membrane potential simultaneously with tension in the femoral artery, in which endothelium-dependent vasodilation appears to depend predominantly, if not solely, on NO. In this vessel the maximum hyperpolarization induced by ACh was only 5 mV in amplitude, typical of NO rather than EDHF (14), and persisted (4 mV) in diabetes. The hyperpolarization and relaxation in the femoral artery were completely blocked by L-NAME.

The incompleteness of the endothelium-dependent relaxation in the femoral artery (43%) is unlikely to reflect additional destruction of NO in the thicker arterial wall, because the relaxation achieved by SNP was also less than maximal (36%). Interestingly, the maximal relaxation evoked by bradykinin in the mesenteric artery was equivalent to that evoked by ACh in the femoral artery; both were submaximal and dependent on NO.

Our observation in femoral arteries that ACh-induced hyperpolarization and relaxation is dependent on NO and entirely resistant to the effects of diabetes supports our conclusion that reduced EDHF rather than NO is responsible for the endothelial vasodilator dysfunction in diabetes. Only one other in vitro study has investigated the impact of diabetes on endothelium-dependent relaxation in a hindlimb vessel of the rat. Taylor and colleagues (16) examined endothelium-dependent responses to ACh in mesenteric and popliteal arteries of rats with diabetes of short duration (3 wk), and they demonstrated impairment of relaxation in both arteries, but blockers were not used on the diabetic arteries to verify the nature of the factor involved. Taylor and colleagues (16) also found no impact of diabetes on endothelium-dependent relaxation to bradykinin in either mesenteric or popliteal arteries, and our observation supports that finding. Furthermore, we also demonstrated that bradykinin-evoked relaxation in arteries from both control and diabetic rats were abolished by L-NAME. We interpret these observations in terms of preservation of the NO-dependent relaxation in STZ-induced diabetes (i.e., ACh-induced relaxation in femoral arteries and bradykinin-induced relaxation in mesenteric arteries) is resistant to the effects of diabetes. In addition, an individual artery in which endothelium-dependent relaxation induced by one agent is impaired during diabetes may be resistant to diabetes with respect to endothelium-dependent vasorelaxation induced by another agent. This study has thus improved our understanding of the relative contributions and deficiencies of the three endothelium-dependent vasodilators in the endothelial vasodilator dysfunction associated with diabetes, at least that which is induced in rats by STZ. Our use of intracellular microelectrodes to measure hyperpolarization and our comparison of two different resistance arterial beds within the same animal has confirmed that the hyperpolarization, the defining characteristic of EDHF, is markedly reduced in this form of diabetes and that this hyperpolarization underpins relaxation. Such understanding is critical to the development of clinically useful intervention strategies to limit the vascular complications of diabetes.

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