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Endothelium-dependent relaxation and noradrenaline sensitivity in mesenteric resistance arteries of streptozotocin-induced diabetic rats

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1 Noradrenaline sensitivity and acetylcholine-induced relaxation were investigated in mesenteric resistance arteries of control and streptozotocin-induced diabetic rats.

2 The diabetic rats demonstrated enhanced vascular sensitivity to noradrenaline compared with age-matched controls (pEC_{50} 5.99 ± 0.06 for diabetic rats, $n = 25$, versus 5.82 ± 0.03 for controls, $n = 45$, $P < 0.05$).

3 Significant impairment of acetylcholine-induced relaxation was observed in arteries from the diabetic animals compared with controls (pEC_{50} 6.81 ± 0.17 for diabetic rats, $n = 21$, versus 7.54 ± 0.17 for controls, $n = 45$, $P < 0.001$).

4 The difference between acetylcholine-induced relaxation in diabetic and control arteries remained in the presence of 10 μM indomethacin (pEC_{50} 6.41 ± 0.11 for diabetic rats, $n = 16$, versus 7.59 ± 0.08 for controls, $n = 20$, $P < 0.001$).

5 The nitric oxide synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA, 1 mM) produced profound inhibition of acetylcholine-induced relaxation in diabetic arteries but partial inhibition in controls. The incomplete inhibition of acetylcholine-induced relaxation by L-NMMA in the control arteries was the result of ineffective inhibition of nitric oxide synthase since an alternative inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 0.1 mM), led to similar inhibition to that seen in the diabetic arteries with L-NMMA. The endothelium-derived relaxing factor (EDRF)-mediated component of acetylcholine-induced relaxation determined by use of the nitric oxide synthase inhibitors was, therefore, apparently reduced in diabetic rats compared with control animals.

6 In further experiments L-NAME was found to enhance the response to noradrenaline in control rats but not in diabetic animals, suggesting that the abnormal response to noradrenaline in the diabetic animals was also due to reduced EDRF release.

7 Nitroprusside-induced relaxation (endothelium-independent) was similar in arteries from control and diabetic rats (pEC_{50} 7.61 ± 0.13 for diabetic arteries, $n = 18$, versus 7.68 ± 0.15 in the controls, $n = 20$, P not significant).

8 These results suggest that endothelial function is abnormal in mesenteric resistance arteries of streptozotocin-induced diabetic rats and that this is predominantly due to reduced EDRF release.

Keywords: Mesenteric resistance arteries; vascular endothelium; vascular smooth muscle; chemically-induced diabetes in rats; streptozotocin; endothelium-derived relaxing factor; nitric oxide synthase inhibitors

Introduction

Vascular disease is a well recognized complication of diabetes mellitus (Christlieb *et al.*, 1976). Alterations in the reactivity of blood vessels to neurotransmitters and circulating hormones have been implicated in the underlying mechanism of microvascular disease and hypertension associated with diabetes (Weidmann *et al.*, 1979). The endothelial cell layer is now established as being an important modulator of vascular smooth muscle tone (Furchgott & Zawadzki, 1980) and abnormal endothelial function has been suggested as a contributary factor in diabetic microvascular dysfunction (Porta *et al.*, 1987). There is one study in human diabetes in which a defect in endothelium-dependent relaxation of the smooth muscle of the corpora cavernosa from diabetic men has been implicated in the high incidence of impotence associated with this disease. The degree of impairment was also found to be related to the duration of diabetes (De Tajada *et al.*, 1989).

In animal models of diabetes there is both histological (Arbogast *et al.*, 1984) and functional evidence that the vascular endothelium is abnormal. In chemically induced diabetes in animals, several studies have demonstrated reduced endothelium-dependent relaxation (Oyama *et al.*, 1986; Pieper & Gross, 1988; Durante *et al.*, 1988; Kamata *et al.*, 1989; Tanz *et al.*, 1989; Mayhan, 1989; Abiru *et al.*, 1990; Tesfamariam *et al.*, 1990; Mayhan *et al.*, 1991) but others have not found any impaired response (White & Carrier, 1986; Wakabayashi *et al.*, 1987; Head *et al.*, 1987; Gebremedhin *et al.*, 1988; Mulhern & Docherty, 1989). Some studies have documented an increase in sensitivity to noradrenaline in arteries from diabetic animals (MacLeod & McNeill, 1985; Harris & MacLeod, 1988; Pieper & Gross, 1988; White & Carrier, 1988; Cohen *et al.*, 1990), but not all (Gebremedhin *et al.*, 1989). It is not established whether this is attributable to abnormal sensitivity of vascular smooth muscle or to reduced relaxing factor production, due to impaired endothelial function.

Most of the *in vitro* studies to date have been investigations of large arteries obtained from diabetic animals and, therefore, have little relevance to the microcirculation or to

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the local control of blood flow or the blood pressure. Two reports have investigated endothelial function in small arteries from the cerebral vasculature in an *in vivo* preparation and have found evidence of dysfunction (Mayhan, 1989; Mayhan *et al.*, 1991). In a recent study resistance artery function of rats has been investigated indirectly by measurement of regional haemodynamics with the technique of Doppler flow (Kiff *et al.*, 1991a,b) and, of the vascular beds investigated, endothelial dysfunction was found in the hindquarters circulation alone (Kiff *et al.*, 1991b). To our knowledge no *in vitro* study has investigated resistance vascular function in small arteries from diabetic animals.

We have, therefore, compared the endothelium-dependent and -independent relaxation, and noradrenaline sensitivity of resistance arteries obtained from streptozotocin-induced diabetic rats and age-matched controls. Using nitric oxide synthase inhibitors and an inhibitor of cyclo-oxygenase we have also attempted to define the role of nitric oxide and prostanoid dilators in endothelium-dependent relaxation in control and diabetic animals.

Methods

Female CSE rats (220–260 g) were injected i.p with streptozotocin (STZ, 56 mg kg^{-1}) in citrate buffer. Control rats were housed separately and both groups given free access to food and water. After 5–6 weeks the animals were killed by cervical dislocation and blood samples obtained by cardiac puncture for glucose determination (glucose oxidase method; YSI Model 23 AM Glucose Analyzer). The mesentery was removed and placed in physiological saline solution (PSS). The solution consisted of (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 25, NaH_2PO_4 1.18, EDTA 0.026 and glucose 5.5, pH 7.4 and bubbled with 5% CO_2/95% O_2.

Small arteries (control internal diameter mean ± s.e.mean; 264 ± 7 μm, $n = 47$; diabetic 284 ± 9 μm, $n = 23$, P not significant) were dissected free from connective tissue using a light microscope and mounted as ring preparations on a small vessel myograph (Mulvany & Halpern, 1977) capable of measuring isometric tension. Arteries were bathed in PSS at 37°C and bubbled with 5% CO_2/95% O_2 and their passive tension and internal circumference were determined. The arteries were set to an internal circumference equivalent to 90% of that which they would have had when relaxed *in situ* under a transmural pressure of 100 mmHg (the maximum active tension for the minimum resting tension is developed at approximately this circumference; Mulvany & Halpern, 1977). In order to obtain arteries of approximately equal diameter in control and diabetic animals the third branch mesenteric arteries were routinely dissected from control rats and the fourth branch dissected from diabetic rats. To assess their contractile responses the arteries were then contracted for 2 min every 10 min on four occasions using the following protocol. The first and fourth contractions were produced with 5 μM noradrenaline in 125 mM potassium solution (KPSS, made by equimolar substitution of KCl for NaCl in PSS). The second was with 5 μM noradrenaline in PSS and the third with KPSS. Any artery failing to produce a maximum active tension equivalent to a pressure of 100 mmHg on the fourth contraction was rejected.

After the routine run-up procedure, the cumulative responses of vessels to noradrenaline were determined (0.05 μM–5.00 μM), the concentration being increased at 3 min intervals. Arteries were then washed three times with PSS and a 15 min washout period allowed before continuation of the protocol.

Arteries were then submaximally contracted with 3 μM noradrenaline for 3 min and relaxation responses to acetylcholine subsequently determined by adding increasing concentrations of acetylcholine at 2 min intervals (final bath concentration 1 nM–10 μM). After a further 5 min indomethacin (10 μM) was added to the bath for 10 min and the arteries again contracted with 3 μM noradrenaline in the continued presence of indomethacin. A second concentration-effect curve to acetylcholine was then determined in the continued presence of indomethacin. After a further 5 min, L-NMMA (N^G-monomethyl-L-arginine, 10 mM) was added to the bath for 10 min and a third acetylcholine concentration-effect curve then determined. After a 15 min recovery period arteries were again pre-contracted with 3 μM noradrenaline and subjected to increasing concentrations of sodium nitroprusside at 2 min intervals (1 nM–10 μM). In order to determine whether noradrenaline contractions were sustained and reproducible throughout the duration of the experimental period, four sequential contractions to 3 μM noradrenaline were performed in the absence of acetylcholine, but in the presence of the inhibitors as appropriate. Similarly, the reproducibility of the acetycholine relaxation was determined by eliciting three consecutive concentration-response curves to acetylcholine in the absence of the inhibitors. These separate time-control studies were carried out on arteries from both diabetic and control populations.

In a further set of experiments the acetylcholine-induced relaxation in arteries from control animals was determined as above but in the presence of the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester, L-NAME (0.1 mM).

A separate investigation into the mode of action of the inhibitor L-NMMA was also carried out in control arteries. Instead of pre-incubating the arteries with L-NMMA, and then determining an acetylcholine concentration-effect curve, arteries were relaxed with acetylcholine (1–100 nM) prior to the addition of L-NMMA (1 mM).

A separate study was also carried out to investigate the role of EDRF in modulating the response to noradrenaline in control and diabetic rat arteries. Seven rats were injected with STZ as above and were killed after 2–3 weeks. Noradrenaline concentration-effect curves were determined as described above. The arteries were then incubated for 10 min with L-NAME (0.1 mM) and the noradrenaline concentration-effect curves repeated in the continued presence of the nitric oxide synthase inhibitor.

Drugs

Chemicals used in this investigation were noradrenaline (Winthrop Laboratories); acetylcholine, indomethacin, sodium nitroprusside, L-NAME, (all from Sigma); L-NMMA (Calbiochem); streptozotocin (gift from Dr Macleod, Upjohn Co., Kalamazoo, U.S.A.). Chemicals were prepared as stock solutions solubilized in PSS except indomethacin which was prepared as a 1 mM stock solution in phosphate buffer (0.02 M KH_2PO_4, 0.12 M $NaH_2PO_4.2H_2O$, pH balanced to 7.8). All concentrations are expressed as the final molar concentration in the organ bath.

Statistical analysis

All values are given as the mean ± s.e.mean. Tension was expressed as mean mN mm^{-1} artery length and the relaxant responses to acetylcholine as a percentage of the initial pre-contraction to noradrenaline. The − log concentration of the drug required to produce 50% of the maximum response (pEC_{50}) was calculated for each concentration-effect curve using the sigmoid equation from the curve fitting programme 'GraphPad' (GraphPad Software Inc., San Diego, CA, U.S.A.). Statistical comparison of the pEC_{50} values for diabetic and control rats was performed with Student's independent t test. Paired t tests were used to compare pEC_{50} values calculated from consecutive concentration-effect curves within control or diabetic artery populations. Where curve fitting was not appropriate the mean values for maximal relaxation between diabetic and control data were analysed by Student's unpaired t test. Significance was assumed if $P < 0.05$.

Results

Five weeks after i.p injection of STZ the plasma glucose concentration was found to be significantly elevated compared with that of the age-matched control animals (45.1 ± 7.5 mmol l^{-1}, in the STZ-treated animals, $n = 15$ versus 7.3 ± 1.1 mmol l^{-1}, in the controls, $n = 14$, $P < 0.001$). Blood glucose levels were not measured in the control animals used for the time-control experiments.

Arteries from diabetic and control rats contracted in a concentration-dependent manner in response to noradrenaline. The concentration-effect curve to noradrenaline was, however, shifted to the left in the diabetic animals, (control arteries pEC_{50} 5.82 ± 0.03, $n = 45$, versus diabetic arteries 5.99 ± 0.06, $n = 25$, $P < 0.05$, Figure 1).

Acetylcholine caused concentration-dependent relaxations in diabetic and control arteries submaximally contracted with noradrenaline, but relaxation was significantly attenuated between 10–100 nM acetylcholine in the diabetic arteries compared with controls (control arteries pEC_{50} 7.54 ± 0.17, $n = 36$; diabetic 6.81 ± 0.17, $n = 21$, $P < 0.001$, Figure 2). Maximum relaxation was, however, not significantly different in diabetic compared with control arteries (Figure 2). Control contractions to noradrenaline without the addition of acetylcholine demonstrated a time-dependent decrease in tension which was not significantly different between diabetic and control arteries.

The addition of indomethacin (10 μM) led to a significant reduction of the noradrenaline pre-contraction in control and diabetic arteries compared with the previous response obtained in the absence of indomethacin (control arteries with indomethacin 2.63 ± 0.20 mN mm^{-1} versus 3.32 ± 0.20 mN mm^{-1}, without indomethacin, $n = 20$, $P < 0.001$; diabetic arteries with indomethacin 3.19 ± 0.25 mN mm^{-1} versus 3.56 ± 0.24 mN mm^{-1}, without indomethacin, $n = 18$, $P < 0.001$). Indomethacin effected no significant change in the subsequent response to acetylcholine in either control or diabetic rats and acetylcholine-induced relaxation remained significantly attenuated in diabetic arteries compared with controls (control arteries in the absence of indomethacin pEC_{50} 7.54 ± 0.16, $n = 36$, versus 7.59 ± 0.08 in the presence of indomethacin, $n = 20$, P significant; diabetic arteries in the absence of indomethacin 6.81 ± 0.17, $n = 21$, versus 6.41 ± 0.11, $n = 16$, in the presence of indomethacin, P not significant, Figure 3a,b). Time-control noradrenaline-induced contractions in the absence of acetylcholine again demonstrated no significant difference in time-dependent relaxation between diabetic and control arteries.

The addition of 1 mM L-NMMA (in the continued presence of 10 μM indomethacin) resulted in an increase in

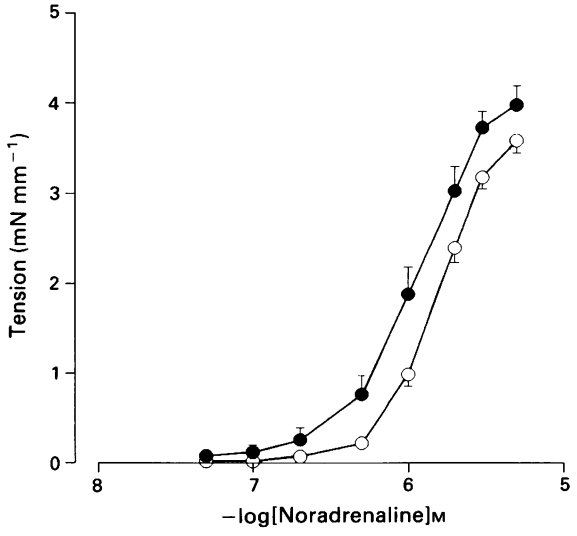

Figure 1 Noradrenaline concentration-effect curves for control (○, $n = 45$) and streptozotocin-diabetic (●, $n = 26$) rat mesenteric resistance arteries.

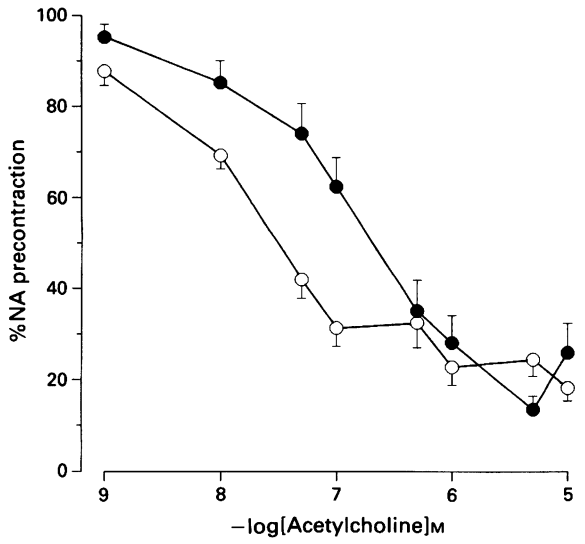

Figure 2 Acetylcholine-induced relaxation after precontraction with 3 μM noradrenaline in control (○, $n = 36$) and streptozotocin diabetic rat mesenteric resistance arteries (●, $n = 24$).

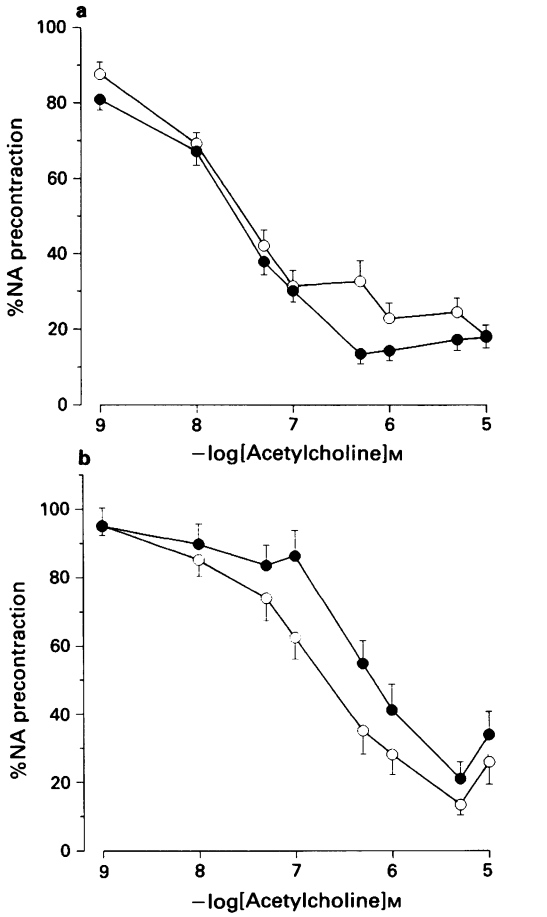

Figure 3 A comparison of the acetylcholine concentration-effect curves after precontraction with 3 μM noradrenaline in arteries taken from (a) control, $n = 20$ and (b) diabetic rats, $n = 18$. Responses were carried out before (○) and after (●) pre-incubation with 10 μM indomethacin.

the noradrenaline pre-contraction in both diabetic and control animals when compared with that obtained in the presence of indomethacin alone (diabetic arteries in indomethacin and L-NMMA, tension = 3.89 ± 0.18 mN mm^{-1} versus 3.19 ± 0.25 mN mm^{-1} indomethacin alone, $n = 18$, $P < 0.005$; control arteries in indomethacin and L-NMMA, tension = 3.42 ± 0.21 mN mm^{-1} versus 2.63 ± 0.20 mN mm^{-1}, in indomethacin alone, $n = 20$, $P < 0.001$). The maximum relaxation to acetylcholine (in the continued presence of indomethacin and L-NMMA) in both diabetic and control arteries was significantly attenuated when compared with the response to indomethacin alone. L-NMMA was, however, more effective as an inhibitor of acetylcholine-induced relaxation in the diabetic arteries which demonstrated 62.6 ± 7.3% inhibition ($n = 18$), whereas control arteries demonstrated only 30.8 ± 5.5% inhibition ($n = 20$, $P < 0.001$, Figure 4a,b). In the time-controls, in the presence of L-NMMA and indomethacin, the precontracted diabetic arteries maintained their initial tension over the duration of the experimental period (percentage change in tension + 0.9 ± 3.2%, $n = 5$) whereas control arteries did relax by a small but significant amount (− 17.5 ± 3.8%, $n = 9$, $P < 0.05$).

The reproducibility of repeated acetylcholine concentration-effect curves was assessed by carrying out three acetylcholine-induced relaxations over the same period involved in the procedures described above. There were no significant differences in the maximal relaxations or pEC_{50} values between the consecutive concentration-effect curves in the arteries from diabetic or control animals.

In order to determine whether the incomplete inhibition of acetylcholine-induced relaxation observed in control arteries in the presence of indomethacin and L-NMMA reflected ineffective inhibition of nitric oxide synthase or the presence of a relaxing factor other than nitric oxide, further experiments were undertaken. Arteries from control rats were subject to the routine 'run-up' procedure and the protocol followed as above but with substitution of the nitric oxide synthase inhibitor, L-NAME (0.1 mM) for L-NMMA (1 mM). The results are presented in Figure 4 (a) which shows a comparison of the acetylcholine response in the presence of indomethacin and either L-NMMA or L-NAME in control arteries. L-NAME effected greater overall inhibition of acetylcholine-induced relaxation than did L-NMMA (L-NAME achieving 69.7 ± 12.0% inhibition, $n = 5$, versus 30.8 ± 5.5% inhibition with L-NMMA, $n = 20$, $P < 0.05$), and the effect was similar to that observed with L-NMMA in the diabetic arteries (L-NMMA effecting 62.6 ± 7.3% inhibition in the diabetic arteries, $n = 18$, compared with 69.7 ± 12.0% inhibition by L-NAME in the controls, $n = 5$, P not significant). In a second set of experiments arteries were pre-contracted with 3 μM noradrenaline and a partial concentration-effect curve to acetylcholine (1–100 nM) determined. L-NMMA (1 mM) was then added to the bath with the subsequent concentrations of acetylcholine (0.5–10 μM) required to complete the concentration-effect curve. The results are shown in Figure 5. Late addition of L-NMMA had a profound effect on acetylcholine-induced relaxation leading to significant constriction relative to the initial tension. (i.e. 5 μM acetylcholine plus indomethacin, tension = 42.83 ± 7.09% of the initial value, $n = 20$; 5 μM acetylcholine plus indomethacin combined with late addition of L-NMMA, tension = 153.5 ± 21.1%, of initial value, $n = 3$, $P < 0.001$).

The responses of arteries pre-contracted with noradrenaline and subjected to increasing concentrations of nitroprusside were similar in control and diabetic arteries (control arteries pEC_{50} 5.68 ± 0.15, $n = 18$, versus 5.61 ± 0.13 in diabetic arteries, $n = 20$, P not significant; Figure 6).

In further experiments, in which rats were studied 2–3 weeks after injection of STZ, the blood glucose values (40.1 ± 6.9 mmol l^{-1}), were comparable to those in rats investigated after 5–6 weeks. In the presence of L-NAME

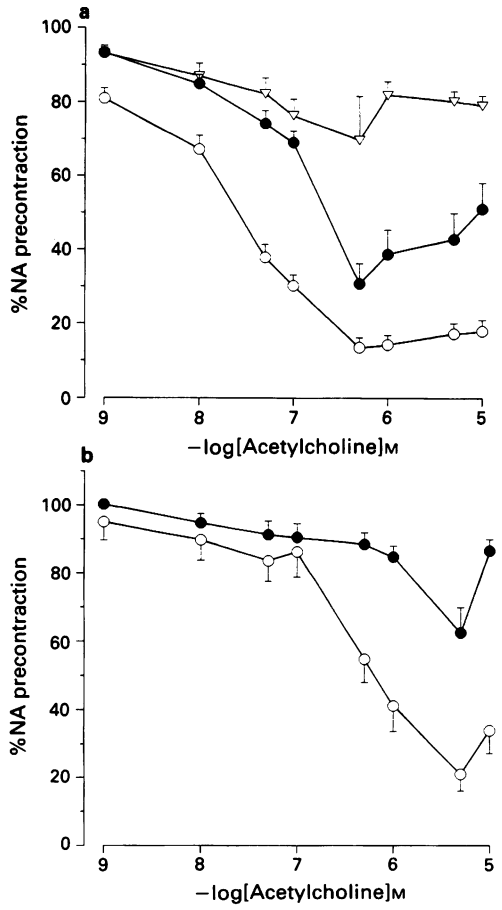

Figure 4 A comparison of the acetylcholine concentration-effect curves after pre-contraction with 3 μM noradrenaline in (a) control; $n = 20$, and (b) streptozotocin-diabetic rat mesenteric resistance arteries; $n = 18$. Responses were carried out in the presence of indomethacin alone (○) and, after pre-incubation in 10 μM indomethacin and 1 mM N^G-monomethyl-L-arginine (L-NMMA) (●). (a) Also includes the acetylcholine concentration-effect curve of a further group of control arteries ($n = 5$) incubated in 10 μM indomethacin plus 0.1 nM L-NAME (▽).

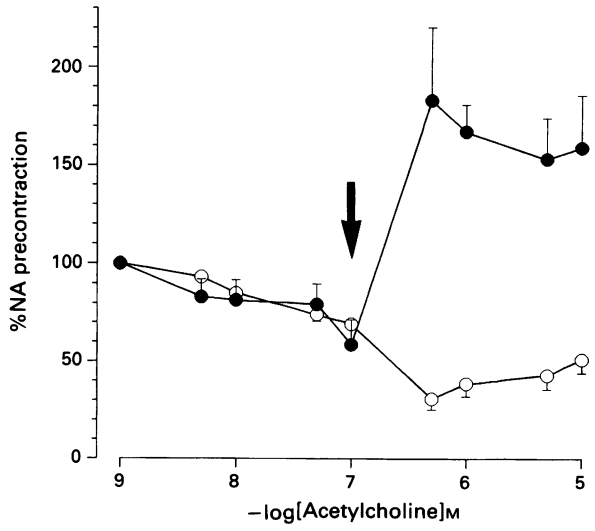

Figure 5 Acetylcholine-induced relaxation in control rat mesenteric resistance arteries demonstrating the profound effect of the bolus addition of N^G-monomethyl-L-arginine (L-NMMA, indicated by the arrow) to partially relaxed vessels (●, $n = 3$) compared with that of vessels incubated for 10 min with L-NMMA prior to exposure to acetylcholine (○, $n = 20$).

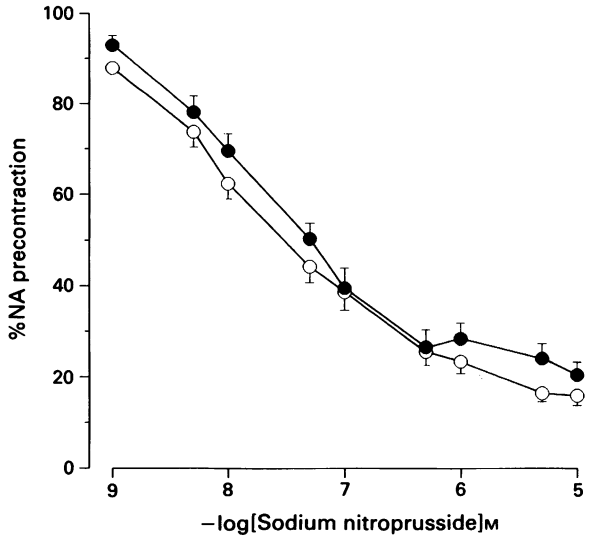

Figure 6 Sodium nitroprusside (SNP) concentration-effect curves for control (○, $n = 20$) streptozotocin-diabetic (●, $n = 18$) rat mesenteric resistance arteries, following pre-contraction with 3 μM noradrenaline.

(0.1 mM) concentration-effect curves to noradrenaline were shifted to the left in arteries from control animals (Figure 7a), but not in those from diabetic rats (Figure 7b; in the absence of L-NAME, control pEC_{50} 5.79 ± 0.08 versus 5.91 ± 0.04, in the presence of L-NAME, $n = 11$, $P < 0.05$; diabetic arteries in the absence of L-NAME pEC_{50} 5.69 ± 0.05 versus 5.70 ± 0.06, in the presence of L-NAME, $n = 14$, P not significant).

Discussion

These results demonstrate enhanced sensitivity to noradrenaline in resistance arteries of rats with STZ-induced diabetes. This enhanced pressor reactivity is in agreement with most studies of conduit arteries in experimental diabetes, the majority of which have investigated chemically-induced diabetes in the rat (MacLeod & McNeill, 1985; Pieper & Gross, 1988; White & Carrier, 1988; Harris & MacLeod, 1988). One study of small arteries found no difference in response to contractile stimuli between cerebral arteries of STZ diabetic rats compared with arteries from control animals (Mayhan *et al.*, 1991). A comparison of the studies is difficult because of the different techniques employed, but the apparent discrepancy between results could be explained by the severity of the diabetes as the rats in this study had higher levels of blood glucose than those of Mayhan *et al.* (1991).

The mechanism for this enhanced noradrenaline sensitivity could be the result of endothelial dysfunction. This was investigated in a further group of diabetic rats and in arteries from control animals. The addition of the nitric oxide synthase inhibitor, L-NAME, potentiated the response to noradrenaline in control arteries, but was without effect in the diabetic animals. This indicates that noradrenaline-induced contractions are normally attenuated by nitric oxide release and agrees with the study of Dohi *et al.* (1990) in which experimental removal of the endothelium in mesenteric resistance arteries of normal rats led to increased reactivity to noradrenaline. This was also suggested by the earlier studies in rat aorta by Martin *et al.* (1986). The lack of effect of L-NAME in the diabetic arteries would suggest a reduced release of nitric oxide. This is in agreement with some, but not all, of the previous investigations of conduit arteries in diabetes. The removal of the endothelium, whilst increasing the contractile response to noradrenaline, did not alter the

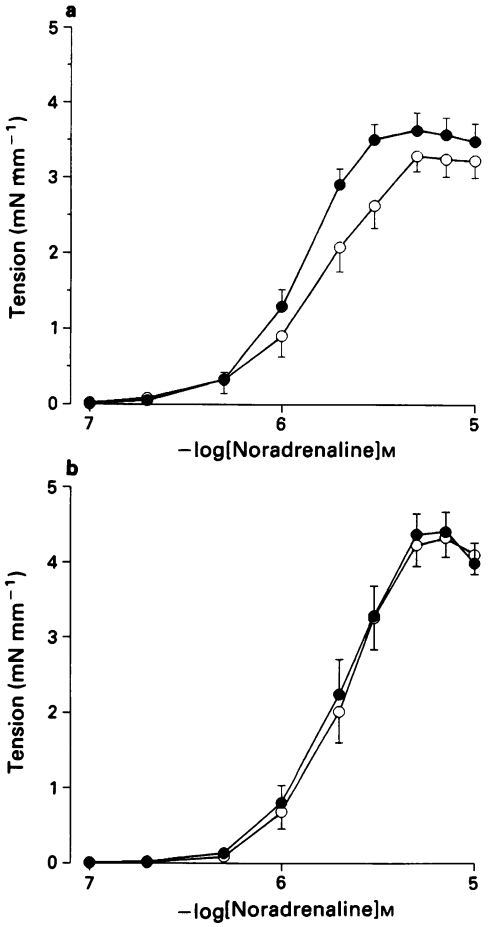

Figure 7 Noradrenaline concentration-response curves in arteries from (a) control rats ($n = 11$) and (b) diabetic rats ($n = 13$) both before (○) and after (●) the addition of N^G-nitro-L-arginine methyl ester (0.1 mM).

enhanced responsiveness to noradrenaline in the arteries of diabetic compared with control rats in studies by Harris & MacLeaod (1988) and by White & Carrier (1988). In contrast, in another study of diabetic rabbits (alloxan-induced) endothelial denudation of control rabbit aorta led to an enhanced response to noradrenaline identical to that seen in diabetic animals (Abiru *et al.*, 1990).

The resistance arteries of diabetic rats demonstrated profound impairment of relaxation to acetylcholine, indicating substantial endothelial dysfunction. This is in agreement with the majority of studies in the conduit arteries of rats with experimentally induced diabetes (Oyama *et al.*, 1986; Pieper & Gross, 1988; Durante *et al.*, 1988; Tanz *et al.*, 1989; Kamata *et al.*, 1989). Studies of cerebral arterioles in the rat (Mayhan, 1989; Mayhan *et al.*, 1991) also showed severe impairment of endothelial function as the responses to ADP and 5-hydroxytryptamine were depressed. Kappagoda *et al.* (1989) found no abnormality in conduit arteries of rats with STZ-induced diabetes, but did in vessels of the BB rat which is genetically susceptible to diabetes, as had been reported by Meraji *et al.* (1987). There is no immediately obvious explanation for the disparities between this study and those showing no impairment of endothelium-dependent relaxation (see Introduction), other than the duration and severity of diabetes which differs from study to study. It is of interest, however, that the data of Harris & MacLeod (1988) initially supported a reduction in sensitivity to acetylcholine in the diabetic rat aorta, but further experiments revealed that this was the result of the very considerable difference in the initial tension (due to the enhanced sensitivity of the diabetic

arteries to noradrenaline). Standardization of tension was found to elicit greater acetylcholine-induced relaxation in diabetic animals than controls. In that study the initial contraction was 50–60% greater in diabetic arteries compared with controls. In the present study there was a small, non-significant difference (<10%) in the initial contraction to noradrenaline but this would be unlikely to account for the very substantial difference in response to acetylcholine.

The results from the present investigation are not in agreement with a recent *in vivo* study by Kiff *et al.* (1991b) which suggested that there was an abnormality of EDRF production in the hindquarters circulation of STZ-treated rats, but not in the mesenteric circulation, which was relatively dilated compared to controls (Kiff *et al.*, 1991a). The studies are not directly comparable as the present experiments were carried out in arteries pre-contracted with noradrenaline. Again there is no obvious explanation for the disparity of results. It is not possible to compare blood glucose concentrations as all values were quoted as being above the upper detection limit of 20 mmol l^{-1} (Kiff *et al.*, 1991a).

Few studies have investigated whether the origin of impaired endothelium-dependent relaxation in diabetic animals lies in abnormalities of the production of different dilator or constrictor stimuli. In this study we hve demonstrated that inhibition of cyclo-oxygenase-dependent pathways by indomethacin had no significant effect on acetylcholine-dependent relaxations in control or diabetic animals, and it is unlikely, therefore, that a dilator prostanoid plays a significant role in acetylcholine-induced relaxation in this vascular bed. In a study of cerebral arterioles in diabetic rats, Mayhan *et al.* (1991) found that indomethacin normalized the reduced dilator response to acetylcholine and suggested that the predominant defect was due to the production of a constrictor prostanoid. Production of a constrictor prostanoid has also been implicated by Tesfarmariam *et al.* (1989) in a study of the aorta in diabetic rabbits. Pre-incubation with the nitric oxide synthase inhibitor, L-NMMA, led to a statistically greater rise in noradrenaline-induced tension in control arteries compared to those from diabetic rats, providing further evidence for reduced nitric oxide production in the diabetic rats.

The response to acetylcholine in the presence of indomethacin and L-NMMA resulted in proportionally greater inhibition of acetylcholine-induced relaxation in the vessels from diabetic rats when compared to controls. This could be interpreted as EDRF playing a greater role in acetylcholine-induced relaxation in the diabetic rats, and would, therefore, imply that the residual acetylcholine-induced relaxation in the control animals was mediated by a compound other than nitric oxide or a prostanoid. This was indeed the interpretation of the data obtained by Dohi *et al.* (1990) who found similar incomplete inhibition of acetylcholine-induced relaxation by L-NMMA and indomethacin in mesenteric resistance arteries from normal Wistar Kyoto rats. However, an alternative explanation to a third component of endothelium-dependent relaxation must be that L-NMMA is selectively ineffective as an inhibitor of nitric oxide synthase in healthy arteries from this vascular bed. In another vascular bed (human subcutaneous fat) we have previously found 1 mM L-NMMA to be an effective inhibitor of acetylcholine-induced relaxation (Woolfson & Poston, 1990), and using the same concentration, this inhibitor has been shown to be as potent as a number of other related compounds tested in rat aorta (Rees *et al.*, 1990). In the mesentery of the control rats in this study the alternative inhibitor L-NAME demonstrated much greater inhibition of nitric oxide synthase than did L-NMMA. L-NAME inhibited acetylcholine-induced relaxation by 69%, thus achieving similar inhibition to that found in the diabetic arteries treated with L-NMMA. An explanation for the different potency of these compounds may lie in their metabolism. Hecker *et al.* (1990) have found L-NMMA to be extensively metabolized in rabbit endothelial cells to L-citrulline and subsequently to L-arginine, whereas another nitric oxide synthase inhibitor, N^G-nitro-L-arginine, was not metabolized (L-NAME was not investigated). During pre-incubation the potential intracellular level of L-NMMA might be quite low if there is very rapid metabolism in the rat mesenteric vascular bed. This hypothesis is supported by the profound and immediate effect of the addition of L-NMMA to control arteries which had been relaxed with acetylcholine. In an earlier study in rabbit aorta, addition of L-NMMA to arteries partially relaxed by acetylcholine was similarly shown to cause immediate inhibition of relaxation, whereas responses of arteries pre-incubated with the inhibitor were inhibited to a lesser extent (Furchgott, 1990). This, it was suggested, could be explained if nitric oxide synthase required stimulation before L-NMMA gained access to, or interacted with it. It could also have been due to rapid metabolism of L-NMMA.

The rate of metabolism of L-NMMA may also provide an explanation for the apparently greater efficacy of L-NMMA in the arteries of diabetic rats as damage to the vascular endothelium might lead to reduced metabolism of L-NMMA. Alternatively, the data could simply reflect reduced acetylcholine-induced release of EDRF (and reduced nitric oxide synthase activity) in the diabetic rats and thus apparently greater efficiency of the inhibitor. Taken together, the data obtained with the two inhibitors L-NMMA and L-NAME suggest that the EDRF-mediated component of acetylcholine-induced relaxation in diabetic rat mesenteric resistance arteries is less than that of the control animals. This has been suggested by one previous study of the diabetic rat aorta in which basal and acetylcholine-induced guanosine levels of cyclic GMP in the vascular smooth muscle were significantly reduced (Kamata *et al.*, 1989). As EDRF leads to vasodilatation through elevation of cyclic GMP, this was indicative of abnormal nitric oxide synthesis. However, that report is in disagreement with another in which cyclic GMP levels were found to be similar in arteries of diabetic and control rats both in the presence and absence of acetylcholine (Harris & MacLeod, 1988).

In this study no attempt has been made to elucidate the mechanisms behind the observed endothelial dysfunction; however, hyperglycaemia itself may play an important role. Elevated glucose concentrations have been found to depress endothelium-dependent relaxation through activation of protein kinase C (Tesfamariam *et al.*, 1991).Together with results from an earlier study in the same laboratory (Tesfamariam *et al.*, 1990) the authors concluded that elevated glucose led to the release of constrictor prostanoids, and thus to inhibition of relaxation.

The reduced endothelium-dependent relaxation in the diabetic animals was unlikely to be the result of impairment of the response of the underlying vascular smooth muscle to EDRF since nitroprusside induced similar concentration-dependent relaxations in vessels from diabetic and control rats. Nitroprusside spontaneously forms nitric oxide in solution and, therefore, provides an estimate of the functional response of the vascular smooth muscle to EDRF (Furchgott & Vanhoutte, 1989). A normal response to nitrovasodilators has also been reported in many studies of conduit arteries from diabetic animals (Durante *et al.*, 1988; Pieper & Gross, 1988; Kamata *et al.*, 1989; Tanz *et al.*, 1989; Tesfamariam *et al.*, 1989).

In conclusion, the resistance arteries in the mesenteric circulation of rats with STZ-induced diabetes demonstrate many of the abnormalities reported in conduit arteries. Since these small arteries are involved in the local control of blood flow and the control of blood pressure, these results indicate that defective endothelial function, manifest by reduced EDRF production may play an important role in the recognized complications of the microvasculature in diabetes.

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