Endothelial function in the isolated perfused mesentery and aortae of rats with streptozotocin-induced diabetes: effect of treatment with the aldose reductase inhibitor, ponalrestat

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1 Noradrenaline sensitivity and relaxation to acetylcholine were investigated in the isolated perfused mesentery and in aortic rings of control and streptozotocin (STZ)-induced (50 mg kg⁻¹) diabetic Charles River rats.

2 In addition, noradrenaline sensitivity and acetylcholine relaxation were similarly assessed in streptozotocin-induced diabetic rats treated from the time of onset of diabetes with the aldose reductase inhibitor, ponalrestat (100 mg kg⁻¹ day⁻¹).

3 The untreated diabetic rats (2–10 weeks after injection of STZ) demonstrated enhanced vascular sensitivity to noradrenaline in the perfused mesenteric arterial tree, compared with age matched controls (pEC₅₀ [–log concentration (M)]: diabetic 5.62 ± 0.09, *n* = 18, *versus* control 5.23 ± 0.07, *n* = 16, *P* < 0.01).

4 Acetylcholine-induced relaxation was significantly impaired in the perfused mesentery of the diabetic animals compared to controls (pED₅₀ [–log dose (mol)]: diabetic 9.87 ± 0.10, *n* = 20, *versus* controls, 10.29 ± 0.09, *n* = 20, *P* < 0.05).

5 In contrast, the aortic ring preparations demonstrated no significant functional differences between the diabetic and control groups in response to either noradrenaline (pEC₅₀: diabetic 7.66 ± 0.08, *n* = 15, *versus* controls 7.55 ± 0.06, *n* = 15, NS), or acetylcholine (pEC₅₀: diabetics 7.30 ± 0.06, *n* = 15, *versus* controls 7.40 ± 0.09, *n* = 15, NS).

6 Treatment with the aldose reductase inhibitor, ponalrestat, did not affect the increased vascular reactivity to noradrenaline, or impaired relaxation to acetylcholine in the perfused mesentery.

Keywords: Mesenteric resistance arteries of rat; rat aorta; vascular endothelium; vascular smooth muscle; chemically induced diabetes; streptozotocin; aldose reductase inhibitors; endothelium derived relaxing factor

Introduction

Abnormal function of the vascular endothelium is now identified with a growing number of conditions associated with arterial disease (Creager *et al.*, 1990; Zeiher *et al.*, 1991; Chwien *et al.*, 1992), including diabetes (Hsueh & Anderson, 1992). There is evidence that impotence in diabetic men may be explained by impairment in the endothelium-dependent relaxation of the smooth muscle of the corpora cavernosa (de Tejada *et al.*, 1989). Other indicators include the demonstration of abnormal relaxation to acetylcholine in the forearm of subjects with non-insulin dependent (type II) diabetes mellitus (NIDDM) (McVeigh *et al.*, 1992), and reduced basal and stimulated nitric oxide (NO) synthesis in patients with insulin-dependent diabetes associated with microalbuminuria (Elliot *et al.*, 1992). In a preliminary report, abnormal endothelium-dependent relaxation to acetylcholine has also been described in resistance arteries from the subcutaneous fat of diabetic subjects without proteinuria (McNally *et al.*, 1992). There is also some evidence that reduced responsiveness of the underlying vascular smooth muscle to NO may contribute to poor endothelial-dependent relaxation (McVeigh *et al.*, 1992; Calver *et al.*, 1992). Indirect evidence for endothelial dysfunction in diabetes includes the observation that the marker of endothelial damage, Von Willebrand's Factor, is elevated in the serum of patients with NIDDM who develop diabetic nephropathy (Stehouwer *et al.*, 1991), and is strongly associated with the development of increased urinary albumin excretion (Stehouwer *et al.*, 1992).

In animal models of diabetes there is histological evidence (Arbogast *et al.*, 1984) that vascular endothelium is abnormal.

Functional studies in conduit arteries from animals with chemically induced diabetes have not been uniform in their findings, but impaired endothelium-dependent relaxation has been described (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; Cameron & Cotter, 1992a), together with increased vascular reactivity to noradrenaline (MacLeod & McNeill, 1985; Harris & MacLeod, 1988; Piper & Gross, 1988; White & Carrier, 1988; Cohen *et al.*, 1990). We have recently reported that resistance arteries from the mesenteric circulation of streptozotocin (STZ)-induced diabetic rats also display grossly impaired endothelium-dependent relaxation to acetylcholine, and an enhanced sensitivity to noradrenaline (Taylor *et al.*, 1992). Using specific inhibitors of NO synthase we demonstrated that both these abnormalities were at least in part due to the reduced production/release of endothelium-dependent relaxing factor.

Amongst the mechanisms that have been proposed to account for diabetic vascular dysfunction, underlying hyperglycaemia is being seen as increasingly central to its aetiology. Several explanations have been put forward which might associate hyperglycaemia with reduced endothelium-dependent relaxation. These include a role for the glucose-stimulated increase in polyol pathway activity and the redox changes associated with increased sorbitol flux (Williamson *et al.*, 1990; Cameron & Cotter, 1992a). Increased sorbitol levels have been identified in aortic endothelium of diabetic rats (Brolin & Naeser, 1988) and could lead to endothelial dysfunction in a number of ways. An increase in sorbitol could compromise endothelial function by a purely osmotic effect (Green & Jaspán, 1990). Increased activity of the polyol

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pathway is also associated with increased utilization of NADPH, which may lead to reduced availability of cellular NADPH, an essential cofactor of NO synthase (Cameron & Cotter, 1992a). Increased polyol pathway activation will also lead to a reduction in glutathione (Dvornik *et al.*, 1987), which in turn may reduce superoxide dismutase activity (Loven *et al.*, 1986). Thus there may be reduced production of NO, together with increased degradation by free radicals. Increased sorbitol flux is also associated with myoinositol depletion which in turn is responsible for a reduction in Na/K ATPase activity (Simmons & Winegrad, 1989). Inhibition of Na/K ATPase can lead to reduced endothelium-dependent relaxation in resistance arteries (Wolfson & Poston, 1991) and could contribute, therefore, to endothelial dysfunction in diabetes.

The rate limiting enzyme in the polyol pathway, aldose reductase, catalyzes the conversion of glucose to sorbitol, which is followed by the conversion of sorbitol to fructose by sorbitol dehydrogenase. There has been considerable interest, therefore, in the possible use of aldose reductase inhibitors (ARI) as a potential means of reversal of the nervous and vascular defects of diabetes. Clinical trials of aldose reductase inhibitors have shown some promise (Sima *et al.*, 1988; Boulton *et al.*, 1990), but have not proven as beneficial as animal studies had intimated (Green & Jaspan, 1990). It has been suggested that the lack of success of ponalrestat, in particular, was due to the inadequate inhibition of aldose reductase achieved with the dose prescribed in clinical trials (Cameron & Cotter, 1992b).

Two recent studies, in conduit arteries, have shown that treatment with an aldose reductase inhibitor (ARI) prevents the development of impaired endothelium-dependent relaxation in STZ-diabetic rat aorta (Cameron & Cotter, 1992a) and alloxan-diabetic rabbit aorta (Tsfamariam *et al.*, 1993). Until now, no study has looked at the effect of ARI treatment on diabetic resistance vascular function. In this study we have investigated the effect of treatment of diabetic rats with ponalrestat, which has shown only very modest benefit in clinical trials (Sima *et al.*, 1988). However, at higher doses, it has recently been shown to prevent nerve conduction velocity deficits in STZ-diabetic rats, which has been ascribed to the improvement of nerve blood flow (Cameron & Cotter, 1992b). In addition, a preliminary report by Chess-Williams & Otter (1993) has also shown that ponalrestat (25 mg kg⁻¹ day⁻¹) prevented endothelial dysfunction in diabetic rat aorta.

In order to ascertain whether a different model of resistance artery function showed the same defect as that we have previously described in isolated mesenteric resistance arteries, we have chosen to use the isolated perfused mesentery (a preparation based on the method of McGregor, 1965) in this study. By way of comparison, we have also investigated constrictor responses and endothelium-dependent relaxation in aortic ring preparations taken from the same animals.

Methods

Animals

Female adult Charles River rats (ZENECA, Alderly Park) were used in this study (220–250 g). Animals were housed separately according to their experimental groups and maintained in a temperature- and light-controlled environment. Food and water were given *ad libitum*.

Induction of experimental diabetes

Diabetes was induced in Charles River rats ($n = 40$) by i.p. injection of streptozotocin, STZ, (50 mg kg⁻¹, ZENECA Pharmaceuticals). Half of the diabetic group were treated daily with the aldose reductase inhibitor, ponalrestat (intraoral administration via a ball-ended stainless steel dosing cannula,

100 mg kg⁻¹ day⁻¹, a dose shown to achieve 95% inhibition of sorbitol accumulation in sciatic nerve tissue of STZ-diabetic rats, ZENECA Pharmaceuticals). Experiments began 2–10 weeks after induction of diabetes with experiments being run in parallel, one rat from each experimental group. All elements of the protocol were carried out on each animal. Blood samples were obtained by cardiac puncture at the time of exsanguination of the animals. Plasma glucose levels were then assayed spectrophotometrically (glucose oxidase analyser).

Isolated perfused mesentery preparation

Vascular reactivity to noradrenaline and the dilator response to acetylcholine were assessed in the isolated perfused mesentery of rats from each of the three groups. The rats were anaesthetized by means of i.p. injection of 'Intraval' (thiopentone, 150 mg kg⁻¹) and cannulated at the superior (anterior) mesenteric artery. The animals were then killed by exsanguination and the mesentery isolated under 'constant flow' perfusion with Krebs solution using a peristaltic pump (6.5 ml min⁻¹, McGregor, 1965). The Krebs buffer was of the following composition (mM): NaCl 120, NaHCO₃ 25, D-glucose 5.5, KH₂PO₄ 1.2, MgSO₄·7H₂O 1.2, KCl 4.7, CaCl₂ 2.5, ethylenediamine-tetra-acetic acid disodium salt (EDTA) 0.026, maintained at 37°C and bubbled with 95% O₂/5% CO₂. Changes in the resistance across the mesenteric arterial tree were measured by monitoring back pressure with a pressure transducer in series between the pump and the preparation. Preparations were allowed to equilibrate for 40 min. Noradrenaline concentration-effect curves were constructed by administration of the drug at a known concentration within the perfusate for 10 min intervals (10⁻⁷–10⁻⁴ M). Following a 20 min washout period, the preparation was precontracted with a supramaximal concentration of noradrenaline. Acetylcholine concentration-effect curves were constructed on top of the noradrenaline contraction through a series of bolus injections, at 2 min intervals, via a self sealing rubber septum (10⁻¹³–10⁻⁸ No of moles). Bolus injections were restricted to small volumes to avoid artifacts (< 4 µl).

Aortic ring preparations

A section of the thoracic aorta between the aortic arch and the diaphragm was dissected out from the same animals following exsanguination. These were mounted as ring preparations and normalized for the measurement of isometric tension in 20 ml organ baths containing Krebs buffer (maintained at 37°C and bubbled with 95% O₂/5% CO₂ throughout). After a standard equilibration time of 40 min under a resting tension of 1 g, during which the Krebs buffer solution was changed every 20 min, cumulative concentration-effect curves to noradrenaline were constructed by addition of small volumes of stock solution to the organ bath (10⁻⁹–3 × 10⁻⁵ M). Contractile responses were expressed as a percentage of the maximum response. Following washout, a 20 min recovery period was allowed before vessels were submaximally precontracted with the required noradrenaline dose to achieve about 80% of the maximum response. When the noradrenaline-induced preincubation reached a plateau level, acetylcholine was added cumulatively as small volumes of stock solution in half log molar increments at 2 min intervals (10⁻⁹–3 × 10⁻⁵ M). Relaxation was expressed as a percentage of the precontraction tension.

Materials

Chemicals used in the study included the following: noradrenaline (Sigma); acetylcholine (Sigma); streptozotocin (ZENECA Pharmaceuticals). Chemicals were prepared as stock solutions solubilized in Krebs, and made daily. Noradrenaline was dissolved with an equal weight of ascorbic acid to prevent oxidation. All concentrations are expressed as

final molar concentrations in the organ bath, except the bolus injection of acetylcholine in the perfused mesentery, which is expressed in terms of the number of moles injected.

Statistical analysis

All values are given as the mean $\pm$ s.e.mean. In both isolated perfused mesentery and aorta, noradrenaline-induced tension is expressed as a percentage of the maximum contractile response and the relaxation to acetylcholine as a percentage of the noradrenaline-induced precontraction. In the perfused mesentery preparations in which acetylcholine was administered as bolus injections, sensitivity to the agonist is expressed in terms of a pED_{50} defined as the negative log of the effective dose (No. of moles) required to produce half the maximum effect. In the aortic ring preparations in which the concentration of the agonist in the bath is known, the negative log of the effective concentration (M) of the drug required to produce 50% of the maximum effect (pEC_{50}) was employed. In either case, the index of sensitivity was calculated from each concentration-effect curve by fitting the data to the sigmoid equation of the curve fitting programme 'GraphPad' (GraphPad Software Inc., San Diego, CA, U.S.A.). One way analysis of variance and independent Student's *t* tests were employed in the statistical comparison of the pEC_{50} or pED_{50} values between groups. The Bonferroni correction was employed for multiple comparisons and significance was assumed if $P < 0.05$ ('InStat' by GraphPad, San Diego, CA, U.S.A.). NS = not significant. Values are given $\pm$ s.e.mean.

Results

Animals injected with STZ experienced significant weight loss compared to age matched controls (mean weight: untreated diabetic 262.1 ± 5.78 g, $n = 21$, versus control 290.0 ± 7.12 g, $n = 22$, $P < 0.01$). Treatment with the aldose reductase inhibitor (ARI) had no significant effect on weight loss in the diabetic animals (mean weight: ARI-treated diabetic 263.6 ± 4.22 g; $n = 22$; versus untreated diabetic 262.1 ± 5.78 g, $n = 21$, NS). Plasma glucose levels were significantly raised in the diabetic animals compared to controls (plasma glucose concentration: untreated diabetic 22.06 ± 3.09 mM, $n = 21$, versus control 4.15 ± 0.31 mM, $n = 22$, $P < 0.001$). No difference was observed in the plasma glucose concentrations between ARI-treated and untreated diabetic animals (plasma glucose concentration: untreated diabetic 22.1 ± 3.1 mM, $n = 21$, versus ARI-treated diabetic 26.8 ± 2.8 mM, $n = 22$, NS).

Noradrenaline reactivity

Cumulative increases in the noradrenaline concentration of the perfusate led to a concentration-dependent increase in the perfusion pressure. The mesentery from the untreated diabetic animals displayed enhanced noradrenaline-sensitivity compared to age-matched controls (pEC_{50} : untreated diabetic 5.62 ± 0.09 , $n = 18$, versus control 5.23 ± 0.07 , $n = 16$, $P < 0.01$). There was no significant difference in the response to noradrenaline between ARI-treated diabetic animals and untreated diabetic animals (pEC_{50} : ARI-treated diabetic 5.69 ± 0.07 , $n = 18$, versus untreated diabetic 5.62 ± 0.09 , $n = 18$, NS) (Figure 1). No significant correlation was observed between the pEC_{50} and duration of diabetes, in either group of diabetic animals.

Aortic ring preparations taken from the same untreated diabetic animals showed no significant change in noradrenaline sensitivity compared to those from control animals (pEC_{50} : untreated diabetic 7.66 ± 0.08 , $n = 15$, versus control 7.55 ± 0.06 , $n = 15$, NS). Moreover, ARI treatment had no significant effect on noradrenaline sensitivity in diabetic rat aortae (pEC_{50} : ARI-treated diabetic 7.71 ± 0.17 ,

Figure 1 Noradrenaline concentration-effect curves for perfused mesentery preparations from control (O, $n = 16$), untreated diabetic (●, $n = 18$) and aldose reductase inhibitor (ARI) treated-diabetic rats (▲, $n = 18$).

Figure 2 Noradrenaline concentration-effect curves for thoracic aortic ring preparations in control (O, $n = 15$), untreated diabetic (●, $n = 15$) and aldose reductase inhibitor (ARI) treated-diabetic rats (▲, $n = 15$).

$n = 15$, versus untreated diabetic 7.66 ± 0.08 , $n = 15$, NS) (Figure 2).

Endothelium-dependent relaxation

In the perfused mesentery preparations, precontracted with noradrenaline, concentration-dependent decreases in perfusion pressure were observed in response to the bolus injections of increasing acetylcholine concentrations. Untreated diabetic rats demonstrated a significantly blunted dilator response to acetylcholine (pED_{50} [$-\log$ dose (No. moles)]: diabetic 9.87 ± 0.10 , $n = 20$, versus control 10.29 ± 0.09 , $n = 20$, $P < 0.05$). The ARI-treated diabetic group demonstrated no significant difference in sensitivity to acetylcholine

Figure 3 Acetylcholine-induced relaxation of isolated perfused mesenteric preparation after precontraction to noradrenaline in control (○, $n = 20$), untreated diabetic (●, $n = 20$) and aldose reductase inhibitor (ARI) treated-diabetic rats (▲, $n = 18$).

Figure 4 Acetylcholine-induced relaxation after precontraction to noradrenaline in thoracic aortic ring preparations from control (○, $n = 15$), untreated diabetic (●, $n = 15$) and aldose reductase inhibitor (ARI) treated-diabetic rats (▲, $n = 15$).

compared to the untreated diabetic group (pED_{50} [– log dose (No moles)]: ARI-treated diabetic 9.90 ± 0.13 , $n = 18$, versus untreated diabetic 9.87 ± 0.10 , $n = 20$, NS) (Figure 3). No significant correlation was observed between the pED_{50} and the duration of diabetes.

In contrast, endothelium-dependent relaxation to acetylcholine in the aortic ring preparations was not significantly affected in the diabetic rats (pEC_{50} : untreated diabetic 7.30 ± 0.06 , $n = 15$, versus control 7.40 ± 0.09 , $n = 15$, NS). In addition, ARI-treatment had no significant effect on acetylcholine sensitivity in the aortae from diabetic rats (pEC_{50} ARI-treated diabetic 7.40 ± 0.06 , versus 7.30 ± 0.06 , $n = 15$ for the untreated diabetic, NS) (Figure 4).

Discussion

The results of this study in the perfused mesentery of rats with STZ-diabetes agree with our previous findings in isolated mesenteric resistance arteries (Taylor *et al.*, 1992) in which we have observed enhanced sensitivity to noradrenaline and impaired endothelium-dependent relaxation to acetylcholine. It is unlikely that these defects are the result of a localized toxic effect of STZ *per se* as similar defects have been demonstrated in alternative models of diabetes, both chemically (Abiru *et al.*, 1990) and genetically-induced (Meraji *et al.*, 1987; Durante *et al.*, 1988; Kappagoda *et al.*, 1989). We previously reported that the observed abnormalities were due, at least in part, to the reduced production and release of nitric oxide. This has been confirmed in a recent study by Lawrence & Brain (1992) which has shown a deficit in the local production of nitric oxide in the cutaneous microvasculature of rats with STZ-induced diabetes.

The absence of these functional defects in the aortae from the same diabetic animals suggests that the resistance vasculature, with its direct relevance to the local control of blood flow, may be more sensitive to the damage associated with the metabolic sequelae of diabetes. This might suggest an underlying difference in the endothelial cell layer between resistance arteries and conduit arteries. Endothelial cells from small vessels do show different functional characteristics from those of larger vessels, for example, glucose may stimulate endothelial cell replication in retinal capillaries but is inhibitory in larger arteries and veins (Porta *et al.*, 1987). However, many previous studies in diabetic animals have shown defects of contractile and endothelial function in conduit arteries. The conflicting results could be explained by the later onset of the abnormalities in the conduit arteries compared to the resistance vasculature. Indeed, in a recent study investigating the time of onset of vascular abnormalities in diabetes, Wong & Tzeng (1992), only observed an enhanced contractile response to noradrenaline in rat aorta 12 weeks after induction of diabetes.

The enhanced sensitivity to noradrenaline seen in this and our previous study in diabetic rats (Taylor *et al.*, 1992) has been documented in several investigations in conduit arteries (MacLeod & McNeill, 1985; Harris & MacLeod, 1988; Pieper & Gross, 1988; White & Carrier, 1988; Cohen *et al.*, 1990) but not all (Head *et al.*, 1987; Gebremedhin *et al.*, 1989; Mulhern & Docherty, 1989). Proposed mechanisms include an increased α_2 -adrenoceptor component of noradrenaline-induced contraction (Scarborough & Carrier, 1983; Wong & Tzeng, 1992) increased constrictor prostanoid production (Agrawal & McNeill, 1987), and reduced EDRF production (Taylor *et al.*, 1992).

The observation of impaired endothelium-dependent relaxation to acetylcholine in this and in our previous investigation (Taylor *et al.*, 1992) agrees with the majority of studies in 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; Cameron & Cotter, 1992a), but several have found no impairment (White & Carrier, 1986; Wakabayashi *et al.*, 1987; Head *et al.*, 1987; Gebremedhin *et al.*, 1988; Mulhern & Docherty, 1989; Kappagoda *et al.*, 1989). The explanation for these discrepant findings is not obvious, but variations in the duration and severity of diabetes and species differences, could be contributory. In addition, the level of antioxidants in the diet of the animals may play a role. Tesfamariam & Cohen (1992), have found that the isolated aortae of rabbits which were pre-fed with an antioxidant (probucol) did not develop abnormal endothelium-dependent relaxation in response to elevated glucose, suggesting that dietary antioxidants may protect against hyperglycaemia-induced oxidative stress.

The primary aim of this study was to determine whether the administration of the aldose reductase inhibitor, ponalrestat, would prevent the development of endothelial dysfunction in rats with STZ-diabetes. As detailed in the

introduction, stimulation of the polyol pathway may lead, theoretically, to endothelial dysfunction through a number of effector pathways. Despite treatment from the onset of diabetes, there was no significant improvement in acetylcholine relaxation or noradrenaline sensitivity in the treated animals compared with those with untreated diabetes over an equivalent period. These results are in contrast with an *in vitro* study in rat aorta by Cameron & Cotter (1992a), in which treatment with a novel aldose reductase inhibitor, (4-amino-2,6-dimethylphenyl-sulphonyl) nitromethane (21 mg kg⁻¹ day⁻¹) over 3 months of STZ-diabetes, completely prevented the development of abnormal endothelium-dependent relaxation to acetylcholine and the calcium ionophore A23187. In the untreated animals, the ratio of maximum contractions to noradrenaline and phenylephrine was enhanced suggesting an increase in α_2 -adrenoceptor-mediated responses. This was partially corrected by treatment with the aldose reductase inhibitor. More recently, Tesfamariam *et al.* (1993) have also found that treatment of diabetic rabbits with a structurally similar aldose reductase inhibitor as that used in this study, zopolrestat (150 mg kg⁻¹), prevented the development of abnormal endothelium-dependent relaxation in aorta, although at a lower dose (50 mg kg⁻¹), zopolrestat did not prevent endothelial-dysfunction. Interestingly, nitric oxide production and vasoconstrictor prostanoid levels were unchanged by the drug (Tesfamariam *et al.*, 1993). The same group have also found that two structurally unrelated aldose reductase inhibitors (sorbitol and zopolrestat) prevented glucose-induced endothelial dysfunction *in vitro* in normal rabbit aorta (Tesfamariam *et al.*, 1992). Therefore, there appears to be a discrepancy between the effect of aldose reductase inhibition on endothelial function in resistance size arteries and conduit arteries in animal models of diabetes. It is unlikely that the duration of treatment provides an explanation as the rats in Cameron & Cotter's study (1992a) were treated over a similar period. Although aldose reductase activity is well described in the endothelium of aorta (Brolin & Naeser, 1988), it is not known whether it is expressed in the endothelial cells of the resistance arteries. Therefore, it is possible that the inhibitor is ineffective due to the absence of the enzyme. Alternatively, although we consider it unlikely,

the dose of ponalrestat used in this study may be ineffective in achieving sufficient inhibition of aldose reductase in the mesenteric arteries. Nonetheless, we can conclude, at least in the circulation studied, that small arteries which contribute to peripheral vascular resistance maintain abnormal endothelial function despite the high dose of aldose reductase inhibitor.

If, as suggested above, the aldose reductase pathway is unimportant in the aetiology of endothelial dysfunction in the resistance vasculature of this animal model of diabetes, then alternative explanations must be sought. A number have been suggested including non-enzymatic glycosylation of proteins (Brownlee *et al.*, 1988; Bucala *et al.*, 1991; Hogan *et al.*, 1992), activation of protein kinase C (Lee *et al.*, 1989; Tesfamariam *et al.*, 1991), increased production of a constrictor prostanoid (Tesfamariam *et al.*, 1990; Tesfamariam & Cohen, 1992) and an increase in free radical production (Pieper *et al.*, 1992; Tesfamariam & Cohen, 1992). A number of recent studies have suggested a particularly important role for glycosylated proteins and increased free radical production, both of which can lead to the effective quenching of nitric oxide (Bucala *et al.*, 1991; Corbett *et al.*, 1992; Pieper *et al.*, 1992). These findings are consistent with the reduced relaxation to nitrovasodilators which we have observed in the diabetic rat mesenteric circulation after prolonged diabetes (Taylor *et al.*, 1994), but not in the short term (Taylor *et al.*, 1992). There is also evidence of reduced nitrovasodilator relaxation in the forearm circulation of diabetic patients (Calver *et al.*, 1992; McVeigh *et al.*, 1992).

In conclusion, the results of the present study are consistent with our previous observations of endothelial dysfunction in the mesenteric circulation in STZ-diabetic rats. The lack of effect of treatment with an aldose reductase inhibitor implies that the polyol pathway does not appear to play a predominant role in endothelial dysfunction in this circulation.

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