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In vivo and *in vitro* evidence of altered nitric oxide metabolism in the spontaneously diabetic, insulin-dependent BB/Edinburgh rat

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1 Altered vasoreactivity may contribute significantly to the pathogenesis of diabetic vascular complications. This study investigated the effect of (a) insulin-treated diabetes, and (b) chronic *in vivo* administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME), a nitric oxide (NO) synthase inhibitor, on mean arterial pressure and *in vitro* vascular reactivity to noradrenaline in mesenteric arterial bed preparations from spontaneously diabetic, insulin-dependent and treated BB rats, the best animal model of insulin-dependent mellitus (IDDM) currently available. Four groups of animals from the Edinburgh colony (BB/E) of spontaneously diabetic BB rats were studied: age-matched (mean ± s.e.mean = 156 ± 2d) non-diabetic (glycated haemoglobin = 3.8 ± 0.1%) and insulin-treated diabetic (glycated haemoglobin = 6.2 ± 0.5%; duration of diabetes = 56 ± 4 d) groups were either L-NAME treated (oral dose = 27 ± 1 mg kg^{-1} d^{-1}; duration of treatment from 30 until 153 days of age) or untreated. Although our diabetic BB/E rats do not achieve overall normoglycaemia, individual adjustment of the daily insulin dose administered to every diabetic rat achieves better glycaemic control than previous groups studying altered vascular reactivity and endothelial dysfunction in this animal model of diabetes.

2 Mean arterial pressure (measured directly via indwelling carotid arterial cannulae) was not significantly different between non-diabetic (116 ± 3 mmHg; $n = 10$) and diabetic (122 ± 2 mmHg; $n = 12$) BB/E rats. L-NAME treatment significantly ($P < 0.001$) increased mean arterial pressure in both groups (165 ± 6 mmHg; $n = 9$ and 142 ± 4 mmHg; $n = 6$ respectively) but the degree of hypertension observed in L-NAME-treated diabetic rats was significantly ($P < 0.01$) attenuated compared to non-diabetic rats treated with L-NAME.

3 Mesenteric arterial bed preparations were cannulated under anaesthesia, excised and intralumenally perfused *ex vivo* with noradrenaline (0.2–20 μM). Basal perfusion pressures were not significantly different in mesentery preparations from non-diabetic (27.0 ± 2.6 mmHg) and diabetic (27.1 ± 3.2 mmHg) BB/E rats. There was no significant difference in maximal response above basal perfusion pressure (MAX) or pEC_{50}, defined as the negative log of the agonist concentration required to give 50% of the maximal response above basal perfusion pressure, to noradrenaline in untreated non-diabetic (166 ± 7 mmHg and 5.74 ± 0.05 respectively) and diabetic (170 ± 11 mmHg and 5.59 ± 0.05) BB/E rats.

4 *In vivo* treatment of non-diabetic and diabetic BB/E rats with L-NAME had no significant effect on basal perfusion pressure (25.9 ± 4.8 mmHg and 28.5 ± 3.9 mmHg respectively). L-NAME treatment *in vivo* increased ($P < 0.001$) MAX to noradrenaline of non-diabetic rats (224 ± 8 mmHg) but did not affect the value for diabetic rats (178 ± 14 mmHg). L-NAME treatment did not alter the pEC_{50} values in either group (5.71 ± 0.05 and 5.65 ± 0.05).

5 Consistent with previous studies using vascular preparations from spontaneously diabetic BB rats, mesentery preparations from diabetic BB/E rats ($n = 12$) exhibited a significantly reduced vasodilator response to acetylcholine (F value = 4.4, $P < 0.05$) across the concentration range studied compared to non-diabetic BB/E rats ($n = 12$) although there was no significant difference in maximal relaxation (diabetic 53.1 ± 4.3% vs non-diabetic 55.7 ± 5.5%) or pEC_{50}, (diabetic 6.92 ± 0.25 vs non-diabetic 7.49 ± 0.22). There was no significant (F value = 0.8, $P > 0.1$) difference in the response to GTN between preparations from non-diabetic and diabetic rats (maximal relaxation: 49.6 ± 3.7% vs 48.5 ± 4.3%; pEC_{50}: 7.84 ± 0.12 vs 7.89 ± 0.22 respectively).

6 In conclusion, vascular responsiveness to noradrenaline is not impaired in spontaneously diabetic BB/E rats with significantly better glycaemic control than those used in previous studies. However, following chronic L-NAME treatment, diabetic BB/E rats exhibit attenuated hypertension and an absence of enhanced vascular responsiveness to noradrenaline *in vitro* compared to similarly treated non-diabetic rats. These results, together with the significantly impaired endothelium-dependent vasodilatation and unchanged endothelium-independent vasodilatation *in vitro* of preparations from diabetic BB/E rats, are consistent with the hypothesis that functional changes in the synthesis and metabolism of NO (rather than altered vascular responsiveness to NO) occur in diabetes. Our results indicate that good glycaemic control alone is insufficient to prevent these abnormalities in NO availability and further studies to characterize the origin of these changes are necessary.

Keywords: Nitric oxide; diabetic BB rat; mesenteric resistance arterial bed; vascular endothelium; vascular smooth muscle

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Introduction

The Diabetes Control and Complications Trial unequivocally demonstrated the importance of intensive insulin treatment and improved glycaemic control in reducing the development and progression of diabetic microvascular disease in insulin-dependent diabetes mellitus (IDDM) (Diabetes Control and Complications Research Group, 1993). Altered reactivity of vascular smooth muscle and impaired endothelium-dependent relaxation may be involved in the pathogenesis of diabetic vascular complications and thus these vascular changes should also be ameliorated or improved by better glycaemic control and intensified insulin therapy. Vascular studies in human subjects indicate that endothelium-dependent vasodilatation is impaired in patients with IDDM *in vivo* (Elliott *et al*., 1993; Johnstone *et al*., 1993) and *in vitro* (McNally *et al*., 1994) and both glucose and insulin have been shown to exert direct effects on vascular reactivity *in vitro* in vessels obtained from non-diabetic animals (Tesfamariam *et al*., 1991; Taylor & Poston, 1994) and from non-diabetic subjects (McNally *et al*., 1995). Due to the inherent difficulties of performing extensive experiments on vascular dysfunction associated with the progression of diabetes in human subjects, two different rat models have been established, the streptozotocin-induced (STZ) diabetic rat and the spontaneously diabetic BB rat.

Studies on vascular reactivity using the STZ diabetic rat have reported inconsistent results with increased, decreased and unchanged responsiveness to noradrenaline (the vasoconstrictor studied most frequently) being reported (Agrawal & McNeill, 1987; Takiguchi *et al*., 1989; White & Carrier, 1990; Cameron & Cotter, 1992; Taylor *et al*., 1992). Although the STZ-diabetic rat is the most commonly used animal model of diabetes, it is not dependent on insulin treatment for survival and therefore few studies have investigated the effect of insulin treatment in these animals which are severely hyperglycaemic. Consequently, the principal explanation for the inconsistences reported relates to the variability of the diabetic state (Tomlinson *et al*., 1992; Poston & Taylor, 1995), a hypothesis which is supported by the prevention of altered vascular reactivity in STZ-diabetic rats treated with insulin (MacLeod, 1985; Takiguchi *et al*., 1989; White & Carrier, 1990).

The spontaneously diabetic BB rat is the best model of IDDM currently available and, in contrast to STZ-diabetic rats but like insulin-dependent diabetic patients, has no detectable circulating immunoreactive insulin and therefore requires daily insulin treatment for survival (Baird, 1989). Studies with diabetic BB rats reported no difference in vascular responsiveness to noradrenaline compared to non-diabetic rats but have consistently observed impaired endothelium-dependent relaxation (Meraji *et al*., 1987; Durante *et al*., 1988; Kappagoda *et al*., 1989; Heygate *et al*., 1995). These results suggest that diabetes-associated endothelial dysfunction, if preventable, requires better control of blood glucose concentration than that achieved in these studies. Several of these studies have been interpreted as evidence for alteration in the metabolism of nitric oxide (NO) and/or the effect of this compound on vascular smooth muscle in diabetes, a hypothesis which is supported by other *in vivo* and *in vitro* studies with human diabetic subjects and STZ-diabetic rats (not treated with insulin) which demonstrated attenuated vascular effects of NO synthase inhibitors (Bucala *et al*., 1991; Taylor *et al*., 1992; Abiru *et al*., 1993; Elliott *et al*., 1993; Sikorski *et al*., 1993). The only study in BB rats to address the role of NO in diabetic vascular dysfunction was an *in vitro* study by Heygate *et al*. (1995) demonstrating impaired relaxation in the mesenteric artery to both acetylcholine and bradykinin which was present in both recent onset and established insulin-treated diabetic BB rats. Since endothelium-dependent vasodilatation, and in particular the NO system, can be a significant determinant of blood pressure, it is surprising that previous studies in the BB rat have not attempted to compare changes in endothelial function *in vitro* with changes in blood pressure in the conscious animals. In this study, we have investigated possible changes in NO synthesis or effects in insulin-treated diabetes by studying (a) the effect of chronic *in vivo* administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor, on both mean arterial pressure and vascular reactivity to noradrenaline *in vitro* and (b) endothelium-dependent and endothelium-independent vasodilatation *in vitro* in isolated perfused mesentery preparations from the Edinburgh colony (BB/E) of spontaneously diabetic BB rats. We consider that these animals provide a unique opportunity for this investigation since good glycaemic control of diabetic BB rats in our colony is achieved by individual adjustment of the daily insulin dose administered to every diabetic animal.

Methods

Animals

A breeding colony of BB rats was established in Edinburgh in 1982 from a small nucleus of animals (3 male and 4 female) kindly donated by Dr P. Thibert from the original BB colony in Ottawa. The BB/E colony consists of two lines created by selectively breeding for and against diabetes and these two lines have now been through 24 generations of strict brother/sister mating on site and have had their inbred status confirmed by skin grafting experiments. All animals are weighed twice weekly from 40 days of age. If they fail to gain weight or lose weight, they are tested for glycosuria (Multistix SG reagent strips, Bayer Diagnostics UK Ltd., Basingstoke, U.K.). If glycosuria is detected, the blood glucose concentration is measured (Exactech blood glucose meter, Medisense Britain Ltd., Birmingham, U.K.) on a blood sample obtained by tail tipping without anaesthesia. In the Edinburgh BB rat colony, a blood glucose concentration of >18 mM is invariably associated with ketonuria, weight loss and the need for daily injection of insulin (Bovine Ultratard U40, Novo Nordisck, Denmark) to survive and these parameters constitute our criterion for classifying an animal as having insulin-dependent diabetes mellitus (IDDM). The daily insulin dose administered to diabetic BB rats is individually adjusted in order to maintain a comparable body weight to age-matched non-diabetic animals. In the high incidence, diabetes-prone mainline (DP BB/E) the incidence of IDDM is 50–60% and the mean ($\pm$s.d.) age at onset of diabetes is 96 ± 18 days. In the diabetes-resistant subline (DR BB/E) the incidence of diabetes is less than 1%. The diabetic and non-diabetic BB/E rats used in this study were all from the diabetes-prone line. All animals were maintained at 20°C on 12 h light/dark cycles and fed Special Diet Services (Witham, U.K.) rat and mouse Number 1 Expanded Feed.

Experimental protocol

Four groups of age-matched (mean $\pm$ s.e.mean $= 153 \pm 2$ d) diabetes-prone BB/E rats were studied: (i) non-diabetic rats not treated with L-NAME ($n = 10$); (ii) diabetic rats not treated with L-NAME ($n = 12$); (iii) non-diabetic rats treated with L-NAME ($n = 9$) and (iv) diabetic rats treated with L-NAME ($n = 6$). The mean duration of diabetes in the diabetic groups was 56 ± 4 d. L-NAME (Sigma Chemical Company Ltd., Poole, U.K.) was dissolved daily in drinking water and treated rats received an oral daily dose of 27 ± 1 mg kg^{-1} body weight from 30 to 153 days of age. The bioavailability of L-NAME administered in this way was confirmed by measuring the mean arterial pressure at 153 days of age. Mean arterial pressure was measured directly in conscious animals via an indwelling carotid arterial cannula

inserted three days previously as follows. Animals were anaesthetised by oral administration of 3% halothane (Fluothane, Zeneca, Cheshire, U.K.). Cannulae were inserted into the carotid artery of each rat and a trochar used to exteriorise the free end of the cannulae which protruded approximately 2 cm at the back of the neck. Each cannula was closed with a small metal pin, the trochar removed and the neck wound sealed with metal clips. Cannulae were flushed with 20 μl heparin (5000 u ml^{-1}) and the rats housed in individual cages to recover from post-operative stress for a 48 h period prior to experimentation. The cannulae were kept patent by daily flushing with a 10% solution of heparin in 0.9% saline.

Isolated perfused mesentery preparation

Rats were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}, i.p.), blood samples collected from the inferior vena cava for measurement of glycated haemoglobin (Glycotest II, Pierce and Warriner (UK) Limited, Chester, U.K.) and plasma glucose concentration (Beckman Synchron CX3 multichannel analyser, Beckman Instruments (UK) Ltd., High Wycombe, U.K.). Mesenteric arterial beds were then isolated from these animals and intralumenally perfused *ex vivo* according to the method of McGregor (1965) as modified by Douglas & Hiley (1990).

Vasoconstriction experiments

Perfusion pressure in response to noradrenaline (0.2–20 μM) was measured with an Elcomatic EM751 transducer (Glasgow, U.K.) and data recorded on a Lectromed Multitrace 2 electronic chart recorder.

Vasodilatation experiments

Endothelium-dependent and endothelium-independent vasodilatation was also studied in mesenteric artery preparations isolated from other groups of non-diabetic ($n=12$) and diabetic ($n=12$) BB/E rats which were matched for age (155 ± 3 d), duration of diabetes (58 ± 5d) and glycaemic control (glycated haemoglobin = 3.7 ± 0.3% and 5.9 ± 0.3% for non-diabetic and diabetic rats respectively, $P<0.001$) with the groups described above. Preparations were preconstricted with noradrenaline (3 μM) and dose-response curves to acetylcholine (10^{-9}–10^{-4} M) and glyceryl trinitrate (GTN; 10^{-9}–10^{-6} M) performed sequentially. The % relaxation was calculated as the percentage decrease in the perfusion pressure observed in the presence of the preconstrictor dose of noradrenaline alone following addition of the vasodilator agent.

The maximal response above basal perfusion pressure (MAX) and the pEC_{50}, defined as the negative log of the agonist concentration required to give 50% of the maximal response above basal perfusion pressure (vasoconstriction experiments) or 50% of the maximal relaxation (vasodilatation experiments), were calculated for each dose-response curve using a commercial curve fitting programme (Biosoft, Cambridge, U.K.).

Statistical analysis

Results are presented as mean $\pm$ standard error of the mean (s.e.mean). Statistical significance of differences between mean dose-response curves were analysed by repeated measures analysis of variance (ANOVA). Student's unpaired t test (population variances not assumed to be equal) was used for all other comparisons.

Results

Biochemical and physiological measurements

There was no significant difference in body weight between the non-diabetic and diabetic groups of BB/E rats studied. Mean values of glycated haemoglobin and plasma glucose concentration (non-fasting) of diabetic rats were significantly ($P<0.001$ and $P<0.05$ respectively) higher than non-diabetic rats (Table 1). Table 1 demonstrates that administration of L-NAME had no significant effect on body weight, glycated haemoglobin or plasma glucose concentration in either group as previously reported (Filep *et al*., 1993). Mean arterial pressures of untreated diabetic and non-diabetic rats were not significantly different but were significantly ($P<0.001$) elevated by L-NAME treatment (Figure 1). The degree of hypertension observed in diabetic rats treated with L-NAME was significantly ($P<0.01$) lower compared to L-NAME-treated non-diabetic rats (Figure 1).

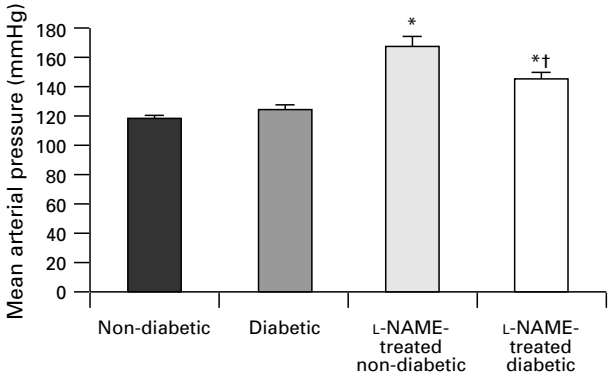

Figure 1 The effect of *in vivo* L-NAME treatment on mean arterial pressure in non-diabetic and spontaneously diabetic BB/E rats. Results are presented as mean $\pm$ s.e.mean. *$P<0.001$ significantly different from untreated group, †$P<0.01$ significantly different from L-NAME-treated non-diabetic rats.

Table 1 Characteristics of non-diabetic and diabetic BB/E rats studied

	Untreated rats		*L-NAME treated rats*	
	Non-diabetic	*Diabetic*	*Non-diabetic*	*Diabetic*
Body weight (g)	327 ± 28	330 ± 17	304 ± 14	313 ± 14
Plasma glucose concentration (nM)	10.0 ± 0.6	13.9 ± 1.4*	10.3 ± 0.6	15.1 ± 1.9*
Glycated haemoglobin (%)	3.8 ± 0.2	6.1 ± 0.5‡	3.9 ± 0.2	6.1 ± 0.5‡
Daily insulin dose (units)	–	2.8 ± 0.1	–	2.8 ± 0.1
Mean arterial pressure (mmHg)	118 ± 3	122 ± 3	165 ± 6¶	142 ± 4†¶
n	10	12	9	6

Data are means $\pm$ s.e. *$P<0.05$, †$P<0.01$, ‡$P<0.001$, significantly different from non-diabetic rats. ¶$P<0.001$, significantly different from untreated group.

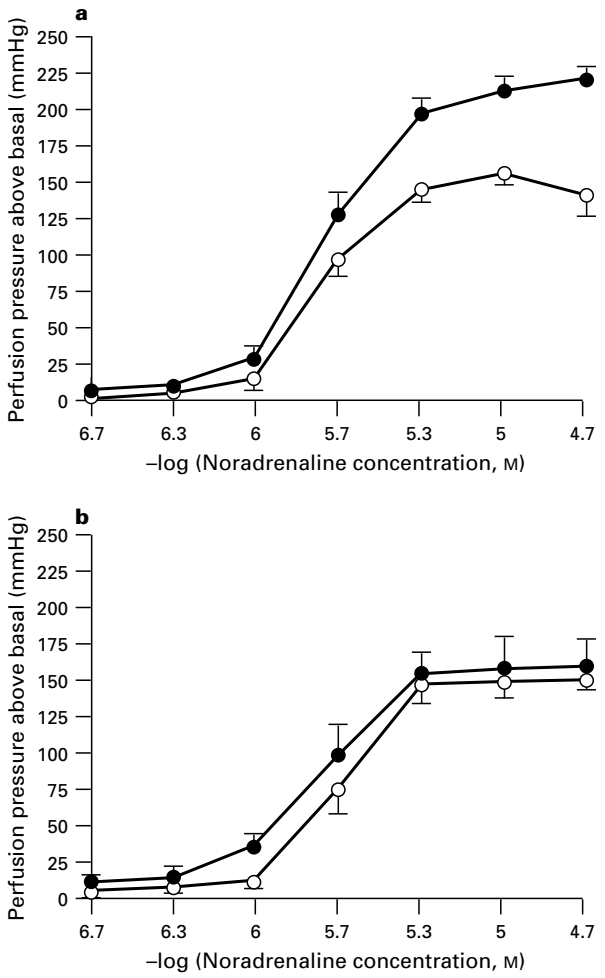

Figure 2 The effect of *in vivo* L-NAME treatment on vascular reactivity to noradrenaline in isolated perfused mesenteric arterial bed preparations from (a) non-diabetic and (b) spontaneously diabetic BB/E rats. Results from untreated (○) and L-NAME treated (●) non-diabetic ($n=10$ and $n=9$ respectively) and diabetic ($n=12$ and $n=6$ respectively) rats are presented as mean ± s.e.mean.

Table 2 Pharmacological parameters derived from noradrenaline dose-response curves of isolated, perfused mesenteric arterial bed preparations from untreated and L-NAME treated non-diabetic and diabetic BB/E rats

	Untreated rats		*L-NAME treated rats*	
	Non-diabetic	*Diabetic*	*Non-diabetic*	*Diabetic*
MAX (mmHg)	166 ± 7	170 ± 11	224 ± 8*	178 ± 14†
pEC_{50}	5.74 ± 0.05	5.59 ± 0.05	5.71 ± 0.05	5.65 ± 0.05
n	10	12	9	6

Data are means ± s.e. $^*P<0.001$, significantly different from untreated non-diabetic group. $\dagger P<0.05$, significantly different from L-NAME-treated non-diabetic group.

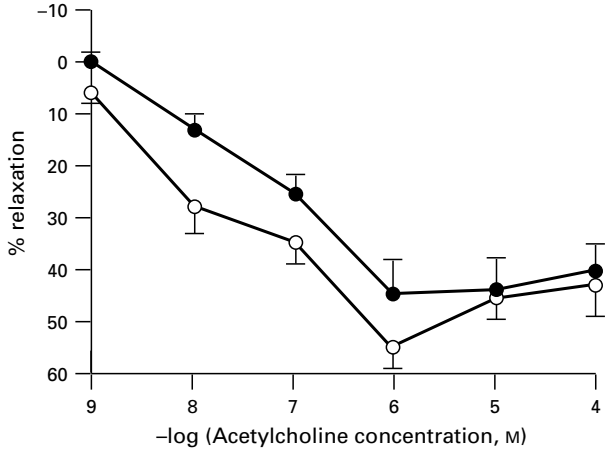

Figure 3 Endothelium-dependent vasodilatation to acetylcholine in isolated, perfused mesenteric arterial bed preparations from non-diabetic and spontaneously diabetic BB/E rats. Abscissa scale shows the concentration of acetylcholine (−log M) and the ordinate scale shows the relaxation expressed as a percentage of the preconstriction perfusion pressure obtained with noradrenaline (3 μM). The responses in the diabetic group (●; $n=12$) are significantly (F value = 4.4, $P<0.05$) different from the responses in the non-diabetic group (○; $n=12$).

Noradrenaline reactivity

Basal perfusion pressures were not significantly different in mesentery preparations from non-diabetic (27.0 ± 2.6 mmHg) and diabetic (27.1 ± 3.2 mmHg) BB/E rats. *In vivo* treatment of non-diabetic and diabetic BB/E rats with L-NAME had no significant effect on basal perfusion pressure (25.9 ± 4.8 mmHg and 28.5 ± 3.9 mmHg respectively). There was no significant difference in the dose-response curves to noradrenaline (F value = 1.8, $P>0.1$) or the mean values of either MAX or pEC_{50} between untreated non-diabetic and diabetic BB/E rats (Figure 2, Table 1). L-NAME treatment significantly increased both the response to noradrenaline across the concentration range studied (F value = 7.3, $P<0.001$) and MAX ($P<0.001$) in non-diabetic rats (Figure 2a, Table 2) but had no significant effect in diabetic rats (F value = 0.7, $P>0.1$; Figure 2b, Table 2). L-NAME treatment did not alter the pEC_{50} values in either group (Table 2).

Endothelium-dependent and endothelium-independent relaxation

Concentration-dependent decreases in perfusion pressure were observed in mesenteric arterial bed preparations submaximally preconstricted with noradrenaline in response to addition of acetylcholine (Figure 3) or GTN (Figure 4). Preparations from diabetic BB/E rats exhibited a significantly reduced vasodilator

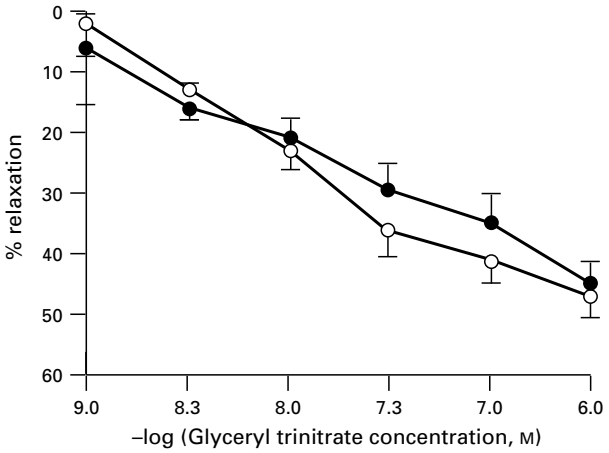

Figure 4 Endothelium-independent vasodilatation to glyceryl trinitrate (GTN) in isolated, perfused mesenteric arterial bed preparations from non-diabetic and spontaneously diabetic BB/E rats. Abscissa scale shows the concentration of GTN (−log M) and the ordinate scale shows the relaxation expressed as a percentage of the preconstriction perfusion pressure obtained with noradrenaline (3 μM). The responses in the diabetic group (●; $n=12$) are not significantly (F value = 0.8, $P<0.1$) different from the responses in the non-diabetic group (○; $n=12$).

response to acetylcholine (F value = 4.4, $P < 0.05$) across the concentration-range studied although there was no significant difference in maximal relaxation (diabetic 53.1 ± 4.3% vs non-diabetic 55.7 ± 5.5%) or pEC_{50} (diabetic 6.92 ± 0.25 vs non-diabetic 7.49 ± 0.22). There was no significant (F value = 0.8, $P > 0.1$) difference in the response to GTN between preparations from non-diabetic and diabetic rats (maximal relaxation: 49.6 ± 3.7% vs 48.5 ± 4.3%; pEC_{50}: 7.84 ± 0.12 vs 7.89 ± 0.22 respectively).

Discussion

In this study we have investigated diabetes-associated abnormalities in NO availability or effects *in vivo* and *in vitro* in the spontaneously diabetic, insulin-dependent BB/E rat, the best animal model of IDDM currently available. Although previous investigations have studied altered vascular reactivity and endothelial dysfunction in BB rats, the degree of glycaemic control achieved in the diabetic animals used in the current investigation is considerably better as demonstrated by both the significantly ($P < 0.01$) lower mean plasma glucose concentration (14.3 ± 1.1 mM; $n = 18$) and the insignificant difference in body weight between the diabetic and non-diabetic rats (Table 1) compared to the values observed in previous studies (Agrawal & McNeill, 1987; Meraji *et al*., 1987; Durante *et al*., 1988; Kappagoda *et al*., 1989). However, despite daily adjustment of insulin dose for each individual diabetic rat, the significantly elevated mean plasma glucose concentration and mean glycated haemoglobin value of the diabetic rats used in this study indicate that these animals do not achieve overall normoglycaemia.

The insignificant difference in vascular responsiveness to noradrenaline in isolated perfused mesentery preparations from non-diabetic and diabetic BB/E rats (Figure 2) is supportive of previous studies using mesenteric arterial and aortic ring preparations isolated from this animal model of diabetes (Agrawal & McNeill, 1987; Meraji *et al*., 1987; Kappagoda *et al*., 1989; Heygate *et al*., 1995). The impaired endothelium-dependent relaxation to acetylcholine in diabetic BB/E rats (Figure 3) is also consistent with previous studies using BB rats (Meraji *et al*., 1987; Durante *et al*., 1988; Kappagoda *et al*., 1989; Heygate *et al*., 1995). Reports of similar impairment of endothelium-dependent vasodilatation in both human diabetic subjects and STZ-diabetic rats have led to the proposal that the production and/or the effects of NO are reduced in diabetes (Cameron & Cotter, 1992; Taylor *et al*., 1992; Abiru *et al*., 1993; Elliott *et al*., 1993; Johnstone *et al*., 1993; Sikorski *et al*., 1993; McNally *et al*., 1994; Otter & Chess-Williams, 1994). The attenuated hypertension (Figure 1) and reduced effect on *in vitro* vascular responsiveness to noradrenaline in diabetic BB/E rats compared to non-diabetic rats following treatment with L-NAME (Figure 2) observed in our study would support this hypothesis. Impaired vasoconstrictor responses to NO synthase inhibition *in vivo* have been reported previously in studies with human IDDM subjects and STZ-diabetic rats (Bucala *et al*., 1991; Abiru *et al*., 1993; Elliott *et al*., 1993). Although previous studies with mesenteric arterial and aortic preparations from STZ-diabetic rats (not treated with insulin) have also demonstrated attenuation (Sikorski *et al*., 1993) and abolition (Taylor *et al*, 1992) respectively of increased vascular responsiveness to noradrenaline following incubation with NO synthase inhibitors *in vitro*, the novel aspect of the current study is that this *in vitro* result was observed following administration of L-NAME *in vivo*. This study also demonstrates for the first time parallel vascular changes *in vivo* as determined by the changes in mean arterial pressure and *in vitro* following chronic NO synthase inhibition. The insignificant difference in endothelium-independent relaxation to glyceryl trinitrate observed in the mesenteric artery preparations isolated from diabetic BB rats in our study confirms that reported previously and suggests that diabetes does not cause a generalized reduction in the sensitivity of vascular smooth muscle to NO (Meraji *et al*., 1987; Durante *et al*., 1988; Kappagoda *et al*., 1989; Heygate *et al*., 1995). This result is also consistent with those of previous reports in STZ-diabetic rats (Hattori *et al*., 1991; Cameron & Cotter, 1992; Taylor *et al*., 1992; Otter & Chess-Williams, 1994) with the exception of Bucala *et al*. (1991). In human diabetic subjects, both unchanged and impaired endothelium-independent vasodilatation has been reported (Elliott *et al*., 1993; Johnstone *et al*., 1993; McNally *et al*., 1994).

Despite the fact that our results may be interpreted as evidence of impaired NO availability in diabetes, the prevention of diabetic vascular dysfunction in STZ-diabetic rats by administration of the NO synthase inhibitors N^G-monomethyl-L-arginine or aminoguanidine suggests that NO production could be elevated in diabetes (Corbett *et al*., 1992; Tilton *et al*., 1993). This hypothesis is supported by the observed increase in NO release by porcine aortic endothelial cells exposed to elevated (44 mM) glucose concentrations (Graier *et al*., 1993). If production of NO is increased in diabetes, an alternative explanation of our data is that the degree of NO synthase inhibition by L-NAME treatment is less in diabetic BB/E rats than in non-diabetic rats. This would be consistent with the relative changes in mean arterial pressure observed in the diabetic BB/E rats treated with L-NAME compared with the non-diabetic rats. Although we have no information on the degree of NO synthase inhibition achieved by the current L-NAME treatment regimen, it seems likely from the dose and duration of treatment used that this would have been significant due to the marked increases in mean arterial pressure observed in the non-diabetic group. L-NAME is also one of the most effective NO synthase inhibitors available and considerably more potent than N^G-monomethyl-L-arginine, the other inhibitor most commonly studied (Gardiner *et al*., 1990; Taylor *et al*., 1992; Filep *et al*., 1993). Although our data could therefore be explained by either a reduced or excess production of NO associated with diabetes, the impaired endothelium-dependent vasodilatation to acetylcholine of mesenteric artery preparations from diabetic BB/E rats suggests that NO synthesis is reduced. Further resolution of this controversy will require direct measurement of NO within different vascular beds.

In conclusion, basal vasoreactivity to noradrenaline is not impaired in spontaneously diabetic BB/E rats with significantly better glycaemic control than those studied previously. However, following chronic L-NAME treatment, diabetic BB/E rats exhibit attenuated hypertension and an absence of enhanced vasoreactivity to noradrenaline *in vitro* compared to similarly treated non-diabetic rats. These results, together with the significantly impaired endothelium-dependent vasodilatation and unchanged endothelium-independent vasodilatation *in vitro* in diabetic BB/E rats, are consistent with the hypothesis that functional changes in the synthesis and metabolism of NO (rather than altered vascular responsiveness to NO) occur in diabetes. Our results indicate that good glycaemic control alone is insufficient to prevent these abnormalities in NO availability and further studies are required to characterize the origin of this change, particularly the relationships with other diabetes-related biochemical abnormalities such as increased polyol pathway activity (Cameron & Cotter, 1992; Otter & Chess-Williams, 1994), oxidative stress (Hattori *et al*., 1991; Keegan *et al*., 1995) and advanced glycation endproduct formation (Bucala *et al*., 1991), to determine the possible role of these changes in the development and progression of diabetic vascular complications.

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