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The effect of insulin treatment and of islet transplantation on the resistance artery function in the STZ-induced diabetic rat

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1 This study was designed to investigate the influence of insulin treatment and islet transplantation on the smooth muscle contractility and endothelium-dependent and independent relaxation of resistance arteries in the chemically induced streptozotocin (STZ) diabetic rat after 6-8 weeks, and 12-14 weeks of diabetes, compared to non-diabetic age-matched controls.

2 The morphology, and contractile responses to high potassium physiological salt solution (KPSS), KPSS containing 10^{-5} M noradrenaline (NAK), and concentration-response curves to noradrenaline (NA) of mesenteric resistance arteries were recorded, along with the endothelium-dependent relaxation responses to acetylcholine (ACh) and bradykinin (BK), and endothelium-independent relaxation to sodium nitroprusside (SNP). Concentration-response curves were then repeated in the presence of a nitric oxide synthase inhibitor, N^G-nitro-L-arginine (L-NOARG).

3 Insulin-treated diabetic rats in the 12 week study demonstrated enhanced vascular contractility to KPSS, NAK and NA, compared to age-matched non-diabetic controls.

4 Incubation with L-NOARG resulted in both a significant increase in maximum contractile response, and sensitivity (pD_2) to NA in the untreated diabetic group (6 weeks). A significant shift in sensitivity was also seen in the insulin-treated diabetic group. In the 12 week study, incubation with L-NOARG resulted in an increased maximum contractile response and sensitivity to NA in the insulin-treated diabetics. An increase in sensitivity was also observed in the untreated diabetic group.

5 Endothelium-dependent relaxation to ACh was significantly augmented in the untreated diabetics (6-weeks), compared to the control group. In the 12-week study, relaxation to both ACh and BK was not significantly different in any of the experimental groups when compared to the sham-operated non-diabetic controls.

6 Incubation with L-NOARG resulted in a significant attenuation of the maximum relaxation response to ACh and BK in all of the experimental groups, in the 6- and the 12-week study.

7 There was no significant difference in the maximum relaxation response or sensitivity to sodium nitroprusside between the diabetic groups and their age-matched controls in either the 6-week or the 12-week study.

8 The results of this study suggest an enhanced release of nitric oxide in the early stages of diabetes, which is more evident in the untreated diabetic rats than the insulin treated, and appears to normalize as the duration of diabetes progresses. This study also shows that the alteration in vascular reactivity of the resistance arteries can be restored to within normal limits by the transplantation of islets of Langerhans, and that islet transplantation is an effective strategy in the correction of the metabolic abnormalities associated with insulin-dependent diabetes.

Keywords: Mesenteric resistance arteries; endothelium; nitric oxide; endothelium derived relaxing factor (EDRF); streptozotocin; islet transplantation; acetylcholine; bradykinin; sodium nitroprusside; N^G-nitro-L-arginine (L-NOARG)

Introduction

Insulin-dependent diabetes mellitus (IDDM) characterized by poor glycaemic control leads to precapillary vasodilatation with increasing blood flow and pressure (Jaap & Tooke, 1995), and this may be the initiating factor in diabetic microangiopathy. Indeed increased regional blood flow and pressure has been shown in the retina (Kohner *et al.*, 1975), kidney (Ditzel & Junker, 1972), and forearm (Halkin, 1991) of diabetic patients.

The endothelium plays an important role in modulating the vascular tone and reactivity of blood vessels (Furchgott & Zawadaski, 1980), by the release of vasoactive agents including prostacyclin, endothelium derived relaxing factor (EDRF), and the vasoconstrictor peptide, endothelin-1 (Yanagisawa *et al.*, 1988); and it has been suggested that abnormal endothelial function may be a contributory factor to large and small vessel disease in diabetes mellitus (Porta *et al.*, 1987). Moreover, vascular smooth muscle from chemically induced diabetic animals appears to be more sensitive to vasoconstrictors and less so to vasodilators (Gebremedhin *et al.*, 1988; Mayhan *et al.*, 1991).

Alteration of vascular reactivity and impaired endothelium-dependent relaxation has been demonstrated in conduit arteries from a number of experimental animal models of diabetes. Attenuated responses to endothelium-dependent vasodilators have been observed in both the spontaneously diabetic Bio Bred (BB) rat (Durante *et al.*, 1988), and the chemically induced diabetes model (Oyama *et al.*, 1986; Mayhan *et al.*, 1991; Cameron & Cotter, 1992). On the other hand, others have reported no change in the response to endothelium-dependent vasodilators (White & Carrier, 1986; Head *et al.*, 1987; Mulhern & Docherty, 1989). Studies of the vascular responses to noradrenaline (NA) have also yielded inconsistent results, showing both an increase in sensitivity to noradrenaline (MacLeod & McNeill, 1985; Cohen *et al.*, 1990), and attenuated responses (Pfaffman *et al.*, 1982; Head *et al.*, 1987).

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There have been comparatively few studies of endothelial function in resistance arteries which reflect the true nature of the microvasculature, and play an important role in the local control of blood flow and pressure. However, impaired endothelium-dependent relaxation has been described in resistance arteries from chemically induced diabetic animals (Taylor *et al.*, 1992; 1994a), and also in the diabetic BB rat (Heygate *et al.*, 1995). Similarly, endothelial dysfunction has been confirmed in studies of human Type 1 (de Tejada *et al.*, 1989; McNally *et al.*, 1994). These observations particularly in the McNally study, support the suggestion that alterations in vascular reactivity may precede the structural changes which are the hallmark of established diabetic microangiopathy.

The introduction of insulin earlier this century removed decompensated diabetes from being the cause of death in IDDM. Subsequently, macrovascular and microvascular disease have been the main causes of morbidity and mortality. Improved glycaemic control, with intensive insulin regimes have reduced the incidence of diabetic microangiopathy (DCCT, 1993). However, optimal diabetic control remains a practical impossibility in many diabetics, since even the most meticulous form of insulin therapy cannot accurately mimic the more precise and subtle blood glucose control afforded by a working pancreas. At present this can be achieved only by the transplantation of islets of Langerhans, or whole pancreas (Orloff *et al.*, 1987; 1988) to replace the function of the damaged beta cells.

In the rat, islet cell transplantation has been shown to reverse metabolic abnormalities, and also has beneficial effects on secondary complications associated with the diabetes (Mauer *et al.*, 1974; Schmidt *et al.*, 1983; Britland *et al.*, 1991). However, to date no studies have investigated whether improved diabetic control following islet transplantation could ameliorate diabetes-induced endothelial dysfunction in the microcirculation.

Accordingly, we have investigated the effects of chemically induced diabetes on resistance artery contractile and relaxation function at six and twelve weeks in rats after the induction of diabetes and observed the response to long-term treatment with either subcutaneous insulin injections, or intra-portal islet transplantation.

Methods

Female WAG/Leicester rats (8–9 weeks old and weighing approximately 150 g) from an inbred colony maintained at the Biomedical Services Department of Leicester University, were used for these studies. In the first study of 6 weeks, rats were randomly divided into three experimental groups: diabetic, control, and insulin-treated diabetic. Diabetes was induced by a singly femoral vein injection of streptozotocin (STZ) 55 mg kg^{-1} dissolved in 1 ml of citrate buffer (0.01 M solution, pH 4.2) under light anaesthesia. Rats allocated to the control group received the buffer solution alone.

Blood glucose levels were measured on a daily basis with a blood glucose test strip (BM-Test 1-44) and a Reflolux meter (Boehringer Mannheim). Rats with blood glucose readings >20 mmol were considered to be diabetic and either received insulin treatment or received no insulin and therefore remained hyperglycaemic for the duration of the study. The insulin-treated group received daily subcutaneous injections of 0.8 u insulin 100 g^{-1} body weight (Ultralente, Novo Nordisk). The dose of insulin was then adjusted in order to maintain euglycaemia according to the following dosing regime: blood glucose 3–4.9 mmol l^{-1} the previous day's insulin dose was halved; blood glucose 5-9.9mmol l^{-1}, no adjustment; blood glucose >10 mmol l^{-1}, dose was increased by 0.15 u insulin.

A second, extended study was performed to investigate the effect of 12–14 weeks of diabetes on the microvasculature. This study consisted of four experimental groups: group 1 animals were injected with STZ and subsequently treated with insulin; group 2 were injected with STZ but did not receive insulin therapy; group 3 were injected with STZ and were treated with insulin for 14–21 days, before receiving an intraportal isograft of freshly isolated pancreatic islets (approximately 1000–1200 per recipient) from syngeneic WAG donor rats (3 donors 1 recipient); and group 4 comprised non-diabetic control rats which underwent a sham islet transplant operation, whereby only the vehicle was injected into the hepatic portal vein.

Pancreatic islets were isolated from male WAG/Leics rats which had been anaesthetized by inhalation of a mixture of halothane, nitrous oxide and oxygen. During this procedure, the pancreatic duct was canulated, and the pancreas distended with collagenase. Islets were then isolated once collagenase digestion had taken place (Sutton *et al.*, 1986), and separated by bovine serum albumin gradient centrifugation (Lake *et al.*, 1987). Islets were incubated in a culture medium overnight at 37°C, prior to hand-picking and transplantation. Reversal of diabetes in the islet-transplanted group was confirmed by three consecutive blood glucose readings being consistently below 10 mmol l^{-1}. Transplanted animals were culled if blood glucose readings were >20 mmol l^{-1}.

In both the six and twelve week studies rats were fed standard rat chow, allowed access to water *ad libitum*, and were weighed daily. Systolic blood pressure was measured by the tail cuff method using plethymography and a physiograph recorder (ITT system), one week before the animal was due to be killed. On the day they were killed insulin-treated diabetic rats did not receive their usual morning injection of insulin. Rats were culled by stunning followed by cervical dislocation. The mesentery and small intestine were removed and placed in a small beaker containing cold physiological salt solution (PSS). The hind limbs were removed at the pelvic joint for tibia length measurement, and the heart excised from the thoracic cavity, blotted dry, and weighed. Rats that had received transplanted islets also had their liver removed and fixed in formalin prior to histological examination and staining for the presence of islets.

Preparation of vessels

Third order vessels (internal diameter <350 μm) were dissected from the mesenteric bed at a point approximately 5 cm from the posterior end of the colon. Two vessel segments of 2 mm in length were mounted in a Mulvany myograph on two 40 μm steel wires. One wire was attached to a force transducer, and the other to a micrometer; this permitted wall tension measurements to be taken at a predetermined internal diameter. Dissection and mounting of the vessels was carried out in cold (4°C) PSS.

Experimental protocol and solutions

After mounting, the vessels were left to equilibrate for 30 min in PSS at 37°C, gassed with 5% CO_2 and 95%O_2 to maintain a pH of 7.4 (PSS composition (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.5, KH_2PO_4 1.0, $CaCl_2$ 2.5, $MgSO_4$ 1.0 and glucose 6). A high potassium physiological salt solution (KPSS) was made by replacing NaCl with KCl. Morphological measurements of media thickness and internal diameter were then made through glass windows in the myograph bath using a water immersion light microscope and a pre-calibrated filar micrometer eyepiece with a resolution of 1 μm. The passive tension and internal circumference of the vessels were determined by a process known as normalisation. Each vessel underwent a series of stretches by adjusting the micrometer. The arteries were then set to 0.9 × $L_{(100)}$ where $L_{(100)}$ is the internal circumference the artery would have *in vivo* when relaxed under a transmural pressure of 100 mgHg (Mulvany & Halpern, 1976). After completing the normalisation procedure, the vessels were maintained in PSS for 1 h before starting the experimental protocol. During this time the bathing medium was changed at 20 min intervals.

The experimental protocol was initiated with three stimulations of KPSS, followed by one stimulation of KPSS con-

taining 10^{-5} M NA. Contractions were maintained for 2 min before rinsing with PSS to base line. After completing the activation procedure, the vessels were rinsed three times and left to recover at baseline for 15 min. Then a cumulative concentration-response curve to NA (10^{-8}–3×10^{-5} M) was performed in the presence of 10^{-6} M cocaine, which was added 20 min prior to the contraction curve to block neuronal reuptake of noradrenaline. The vessels were then rinsed to baseline and allowed to recover for 15 min.

The arteries were then maximally contracted with a bolus dose of NA10^{-5} M and once a plateau had been reached, the relaxation response to acetycholine (ACh 10^{-9}–10^{-5} M) observed. Fifteen minutes after washout, arteries were maximally contracted again with NA (10^{-5} M), and were subsequently relaxed with cumulative doses of bradykinin (BK, 10^{-9}–10^{-5} M). The vessels were brought to base line with three rinses of PSS, and incubated with PSS containing 10^{-5} M N^G-nitro-L-arginine (L-NOARG) for 1 h. The NA, ACh, and BK concentration-response curves were repeated in the presence of L-NOARG. Finally, the vessels were maximally contracted with NA (10^{-5} M) and relaxed with cumulative concentrations of sodium nitroprusside (SNP 10^{-9}–10^{-4} M). At the end of each study, all vessels were rinsed in calcium-free PSS before fixing overnight in 10% formalin for histological examination.

Drugs

All drugs were freshly prepared on the day of study and dissolved in distilled water, with the exception of the streptozotocin which was made up in citrate buffer. The STZ was dissolved immediately prior to injection in saline acidified to pH 4.2 with citric acid (0.01 M). Streptozotocin, noradrenaline hydrochloride, cocaine hydrochloride, acetylcholine chloride, bradykinin, sodium nitroprusside, and L-NOARG were obtained from Sigma Chemical Company, Poole, Dorset, UK.

Data and statistical analysis

Results are expressed as the mean ± standard error of the mean (s.e.mean). Contractile responses to NA are expressed as active tension (mN mm^{-1}), which is calculated from the measured force divided by twice the vessel length. Relaxation responses to ACh, BK, and SNP are expressed as a percentage decline of the maximum contractile response. The sensitivity (EC_{50}) i.e. the concentration of the drug required to produce 50% of the maximum response, was calculated by computer-aided curve fitting programme for NA, ACh, BK and SNP concentration-response curves. The EC_{50} values were then converted to $-\log EC_{50}$s or pD_2s, and parametric statistical tests were applied. Multiple comparisons were firstly made between means of the experimental groups by a one-way analysis of variance (ANOVA). If $P<0.05$, individual pairs of means were then compared by the Bonferroni multiple comparisons test. Comparisons within groups were determined by the Student's paired t test.

Results

Morphology

There were no significant differences in media lumen, media volume, media/lumen ratio or vessel diameter between the experimental groups in the six week or the twelve week study (Table 1)

Physical characteristics of diabetic animals (Table 2)

Six week study Insulin-treated rats were significantly heavier than the untreated diabetic rats (194 g ± 6 versus 174 g ± 4, $P<0.05$, $n=8$), although they were not significantly heavier than the control non-diabetic rats (177 ± 8, $n=5$), and the body weight of the untreated diabetic rats was not significantly different from that of the controls. Heart weight/tibia ratio was significantly higher in diabetic rats treated with insulin compared to non-diabetic controls (2 ± 0.12 versus 1.68 ± 0.08, $P<0.05$). Blood glucose levels were significantly greater in the untreated diabetic group (18.4 ± 2.2 mmol l^{-1}, $n=8$) compared to the control group (3.1 ± 0.2 mmol l^{-1}, $n=9$ $P<0.001$), or those receiving insulin (9.13 ± 2.48 mmol l^{-1}, $n=7$ $P<0.01$). The diabetic animals receiving insulin also had significantly higher blood glucose levels than the control animals ($P<0.01$). There was not significant difference between blood pressure of the experimental groups.

Table 2 Physical characteristics of STZ-induced diabetic rats, insulin-treated STZ rats, islet-transplanted rats and their age-matched controls

	Weight (g)	*Heart wt. /tibia*	*Blood pressure* (mmHg)
6-wk Control	177 ± 8 ($n=5$)	1.68 ± 0.08 ($n=7$)	139 ± 4 ($n=8$)
6-wk Diabetic	174 ± 4** ($n=8$)	1.73 ± 0.03 ($n=8$)	139 ± 7 ($n=6$)
6-wk Diabetic + insulin	194 ± 6 ($n=8$)	2 ± 0.12* ($n=6$)	136 ± 3 ($n=6$)
12-wk Control	214 ± 4 ($n=10$)	1.71 ± 0.05 ($n=10$)	124 ± 3 ($n=4$)
12-wk Diabetic	152 ± 7*†† ($n=9$)	1.46 ± 0.05† ($n=8$)	133 ± 6 ($n=8$)
12-wk Diabetic + insulin	210 ± 14 ($n=8$)	1.83 ± 0.08 ($n=8$)	125 ± 4 ($n=7$)
Islet-transplanted	187 ± 7* ($n=8$)	1.71 ± 0.06 ($n=7$)	132 ± 2 ($n=8$)

*$P<0.05$ (unpaired t test versus control); **$P<0.01$ (unpaired t test diabetic versus diabetic + insulin); †$P<0.05$ (unpaired t test versus control, diabetic + insulin, and transplanted animals); ††$P<0.01$ (unpaired t test diabetic versus transplant)

Table 1 Morphological measurements of vessels of STZ-induced diabetic rats, insulin-treated diabetic rats, islet-transplanted rats and age-matched controls

	Diameter (μm)	*Media thickness*	*Media Vol.*	*Medial lumen*
6-wk Control ($n=9$)	262 ± 8	11.82 ± 0.81	10146 ± 847	4.58 ± 0.37
6-wk Diabetic ($n=8$)	263 ± 6	10.23 ± 0.44	8741 ± 388	3.94 ± 0.22
6-wk Diabetic + insulin ($n=8$)	249 ± 4	12.22 ± 0.63	9996 ± 532	5 ± 0.3
12-wk Control ($n=12$)	250 ± 6	12.64 ± 0.67	11337 ± 762	4.92 ± 0.32
12-wk Diabetic ($n=9$)	254 ± 10	14.62 ± 1.22	12419 ± 1094	6.03 ± 0.87
12-wk Diabetic + insulin ($n=8$)	257 ± 7	15.14 ± 1.1	12784 ± 1001	6.15 ± 0.55
Islet-transplanted($n=8$)	261 ± 9	14.1 ± 0.5	12208 ± 396	5.48 ± 0.33

Twelve week study The body weight of the untreated diabetic animals was significantly lower than that of the remaining three experimental groups (152 g±7 versus control 214 g±4, $P<0.001$; insulin-treated diabetics 210 g±14, $P<0.01$; and transplanted animals 187 g±7, $P<0.01$). The transplanted animals were also significantly lighter than the control group (187 g±7 versus 214 g±4, $P<0.01$). The untreated diabetic animals developed cataracts in both eyes by the final week of the twelve week study. Heart weight/tibia ratio was significantly different in the untreated diabetic animals compared to the other experimental groups (1.46±0.05 versus control 1.71±0.05, $P<0.05$; insulin-treated diabetics 1.83±0.08, $P<0.001$; and the transplanted group 1.71±0.06, $P<0.05$). At the end of the study, blood glucose levels were significantly greater in the untreated diabetic group (24.2±1.1 mmol l^{-1}, $n=8$, $P<0.001$) compared to those receiving insulin (13.4±0.7 mmol l^{-1}, $n=8$), and the control animals (4.4±0.67 mmol l^{-1}, $n=6$). Blood glucose was significantly lowered from hyperglycaemic levels to the normal range approximately ten days after islet transplantation (pre-transplant: 16.4±1 mmol l^{-1} versus post-transplant: 4.8±0.6 mmol l^{-1}, $n=8$, $P<0.0001$). There was no significant difference between the blood pressure of the experimental groups.

Contraction studies

Six week study In the six week study, there was no significant difference in maximum contractile response to KPSS (control; 3.22±0.16 mN mm^{-1}, $n=9$; untreated diabetic: 3.43±0.18 mN mm^{-1}, $n=8$; insulin-treated diabetic: 3.35±0.26 mN mm^{-1}, $n=8$), KPSS containing NA 10^{-5} M (NAK) (control: 4.12±0.19 mN mm^{-1}, $n=9$; untreated diabetic 4.25±0.34 mN mm^{-1}, $n=8$; insulin-treated diabetic: 4.3±0.34 mN mm^{-1}, $n=8$), or to NA between the groups (control: 3.89±0.17 mN mm^{-1}; untreated diabetic: 3.85±0.28 mN mm^{-1}; and insulin-treated diabetic: 3.79±0.29 mN mm^{-1}). The NA sensitivity (pD_2) was also unchanged between the groups (control: 5.77±0.03; untreated diabetic: 5.74±0.01; and insulin-treated diabetic: 5.8±0.01).

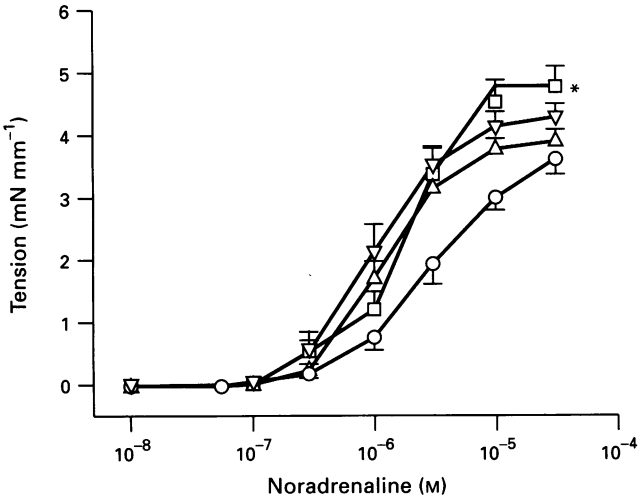

Figure 1 Contractile response to noradrenaline of rat mesenteric resistance vessels (12 week study) from sham-operated non-diabetic rats (○), insulin-treated diabetic rats (□), untreated diabetic rats (△), and islet-transplanted rats (▽). Points show mean±s.e.mean.

Twelve week study In the twelve week study, both the contractile responses to KPSS, and NAK^+ were significantly increased in the insulin-treated diabetic group (KPSS: 4.43±0.28 mN mm^{-1}, $P<0.05$; and NAK: 5.55±0.34 mN mm^{-1}, $P<0.05$, $n=8$) compared to the controls (KPSS: 3.2±0.24 mN mm^{-1}; NAK: 4.19±0.31, $n=12$), untreated diabetics (KPSS: 3.34±0.13 mN mm^{-1}; NAK: 4.13±0.19 mN mm^{-1}, $n=8$), and the transplanted animals (KPSS: 3.11±0.25 mN mm^{-1}; NAK 4.22±0.31 mN mm^{-1}, $n=8$). Also, the maximum contractile response to NA was significantly greater in the insulin-treated group (4.82±0.33 mN mm^{-1}, $P<0.05$, $n=7$) compared to the other three experimental groups (controls: 3.9±0.26 mN mm^{-1}; untreated diabetic: 3.96±0.2 mN mm^{-1}; transplants: 4.38±0.3 mN mm^{-1}), although there was no change in the NA sensitivity (insulin-treated: 5.8±0.08; control: 5.99±0.07; untreated diabetic: 6.03±0.08; transplants: 6.04±0.08) (Figure 1, Table 3).

Effects of L-NOARG on the NA contractile response and sensitivity

Six week study Incubation with the nitric oxide synthase inhibitor, L-NOARG resulted in a significant increase in maximum NA contractile response in the untreated diabetic group ($P<0.05$), along with a significant shift in the sensitivity to NA (pD_2) (Figure 2a). There was no significant increase in either the maximum contractility or sensitivity to NA in the control group (Figure 2b). The maximum contractile response also remained unaltered in the insulin-treated diabetic group, although there was evidence of a significant shift in sensitivity ($P<0.05$) (Figure 2c, see Table 3). The percentage shift after the addition of L-NOARG was also calculated within each group by the following formula:

$$-\frac{(pD_2)-(pD_2+L-NOARG)}{pD_2}\times 100$$

An analysis of variance (ANOVA) was then carried out between the experimental groups. As a result, there was no significant shift evident between the groups (control: 1.35±0.41%, $n=9$; untreated diabetic: 0.95±0.3%, $n=8$; insulin-treated diabetic: 1.42±0.47%, $n=8$).

Twelve week study Incubation with L-NOARG resulted in an increase in both maximum contractile response and sensitivity to NA in the insulin-treated diabetics ($P<0.05$) (Figure 3a). In contrast to the findings of the six week study, the untreated diabetics showed no significant increase in contractility, although an increase in sensitivity (pD_2) was evident ($P<0.05$)

Table 3 Contractile responses and sensitivity to noradrenaline before and after incubation with N^G-nitro-L-arginine (L-NOARG) in six and twelve week STZ study

	NA Max.	*+L-NOARG*	*pD_2*	*+L-NOARG*
6-week Control ($n=9$)	3.89±0.17	3.85±0.22	5.77±0.03	5.8±0.02
6-week Diabetic ($n=8$)	3.85±0.28*	4.32±0.33	5.74±0.01*	5.79±0.02
6-week Diabetic+insulin ($n=8$)	3.79±0.29	3.95±0.35	5.8±0.01*	5.87±0.02
12-week Control ($n=12$)	3.9±0.26	3.82±0.23	5.99±0.07	6.28±0.17
12-week Diabetic ($n=8$)	3.96±0.2	3.82±0.19	6.03±0.08*	6.27±0.07
12-week Diabetic+insulin ($n=7$)	4.82±0.33*	5.26±0.39	5.8±0.08*	6.25±0.07
Transplants ($n=7$)	4.38±0.23	4.33±0.24	6.04±0.08	6.27±0.08

*$P<0.05$ (paired t test before and after L-NOARG).

(Figure 3b). However, the contractility and sensitivity in both the control and the islet-transplanted group were not significantly different after incubation with L-NOARG (Table 3). Similarly, the shift in sensitivity was calculated for each group using the aforementioned formula, and an ANOVA was carried out between all of the experimental groups. This showed that there was no significant difference in the percentage shift evident between the experimental groups (control: $7.01 \pm 1.51\%$, $n=10$; untreated diabetic: $5.19 \pm 1.02\%$, $n=8$; insulin-treated diabetic: $7.69 \pm 2.33\%$, $n=7$; transplants: $4.89 \pm 1.29\%$, $n=8$).

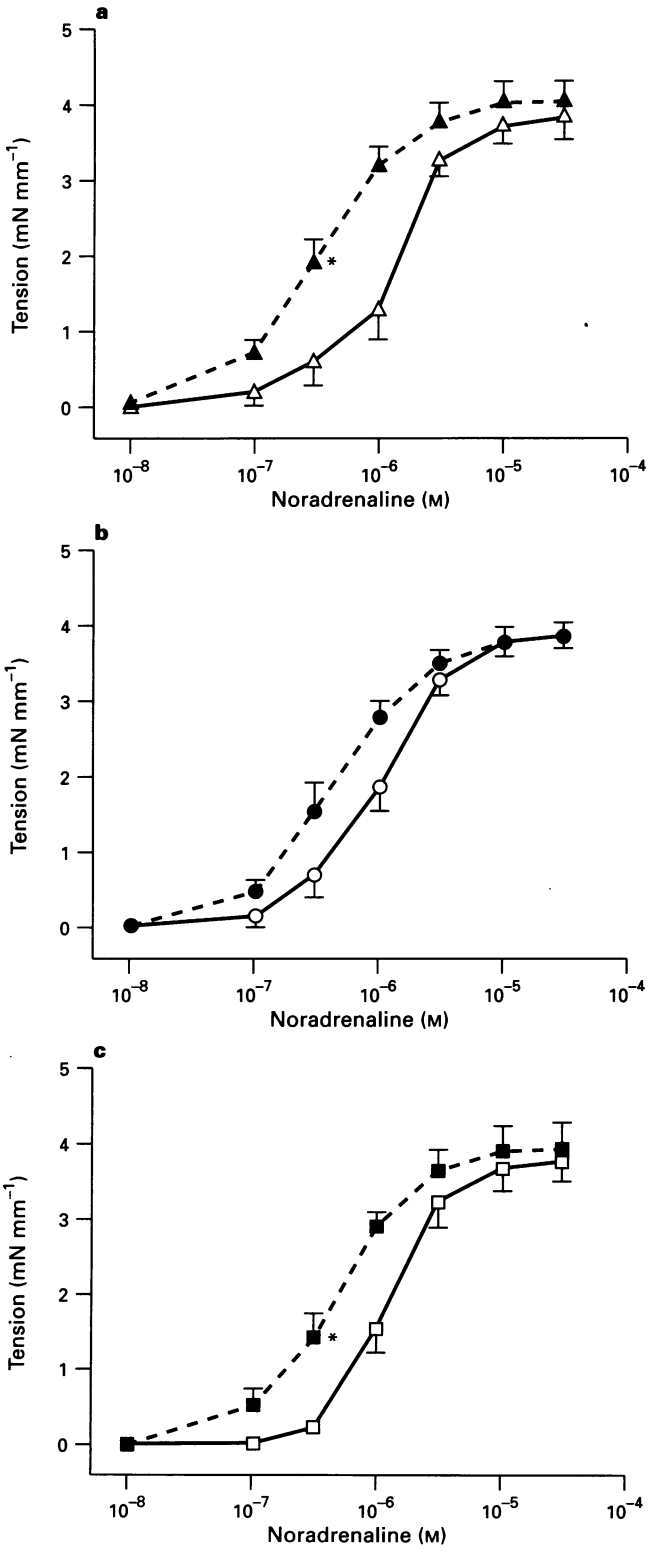

Figure 2 Contractile response to noradrenaline of rat mesenteric resistance vessels (6 week study) before and after incubation with N^G-nitro-L-arginine (L-NOARG) taken from (a) untreated diabetic rats (△); + L-NOARG (▲); (b) controls (○); + L-NOARG (●); and (c) insulin-treated rats (□); and + L-NOARG (■). Points show means ± s.e.mean.

Acetylcholine relaxation responses

Six week study Maximum relaxation to the endothelium-dependent vasodilator ACh was significantly greater in the untreated diabetic group compared to the control group ($92 \pm 1\%$, $n=7$ versus $64 \pm 10\%$, $n=8$, $P<0.05$), although there was no significant difference in sensitivity between the groups (control: 6.9 ± 0.11; untreated diabetic: 6.96 ± 0.1; insulin-treated diabetic: 6.99 ± 0.08). Relaxation in the insulin treated-diabetic group was not significantly greater than that seen in the control group ($79 \pm 7\%$, $n=8$ versus $64 \pm 10\%$) (Figure 4a).

Incubation with the nitric oxide synthase inhibitor L-NOARG caused significant attenuation of the maximum relaxation response to ACh in all three experimental groups ($P<0.05$) with the untreated diabetic group displaying the least inhibition with L-NOARG. However, there was no change in ACh sensitivity (pD_2) in any of the three experimental groups (Table 4).

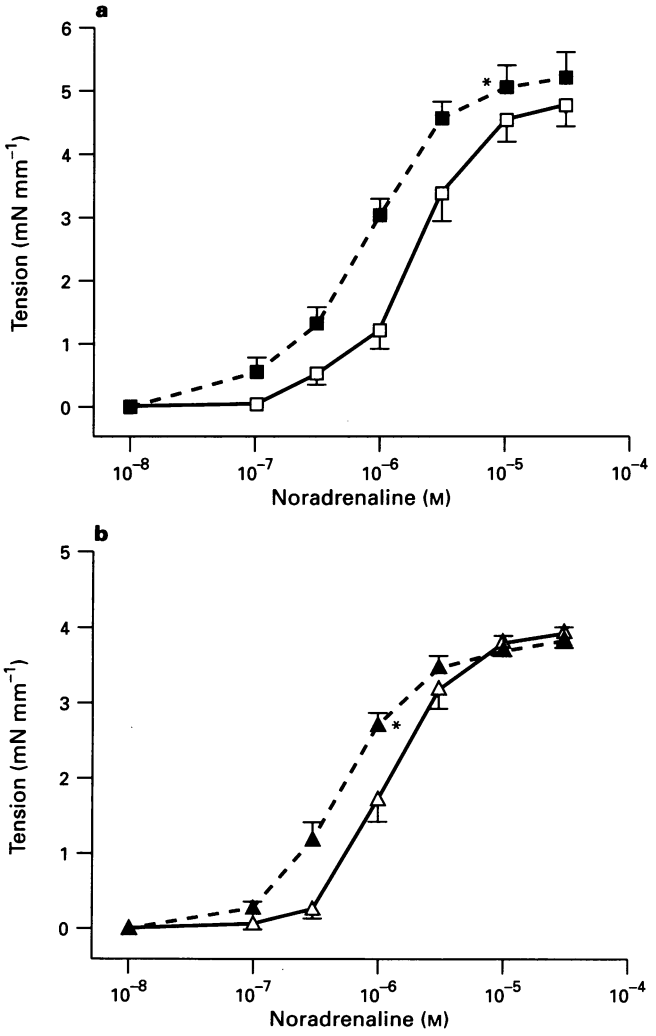

Figure 3 Contractile response to noradrenaline of rat mesenteric resistance vessels (12 week study) before and after incubation with N^G-nitro-L-arginine (L-NOARG) taken from (a) insulin treated rats (□); + L-NOARG (■); and (b) untreated diabetic rats (△); and + L-NOARG (▲). Points show means ± s.e.mean.

Twelve week study There was no significant difference in ACh maximum relaxation between the experimental groups and the sham-operated non diabetic controls (untreated diabetics: 84±3%, $n=8$; insulin-treated diabetics 75±5%, $n=8$; and transplants: 72±7%; versus controls: 86±3%, $n=10$) (Figure 4b). The ACh sensitivity remained unaltered with no significant difference between the experimental groups (control: 7.1±0.07; untreated diabetic: 7.1±0.1; insulin-treated dia-

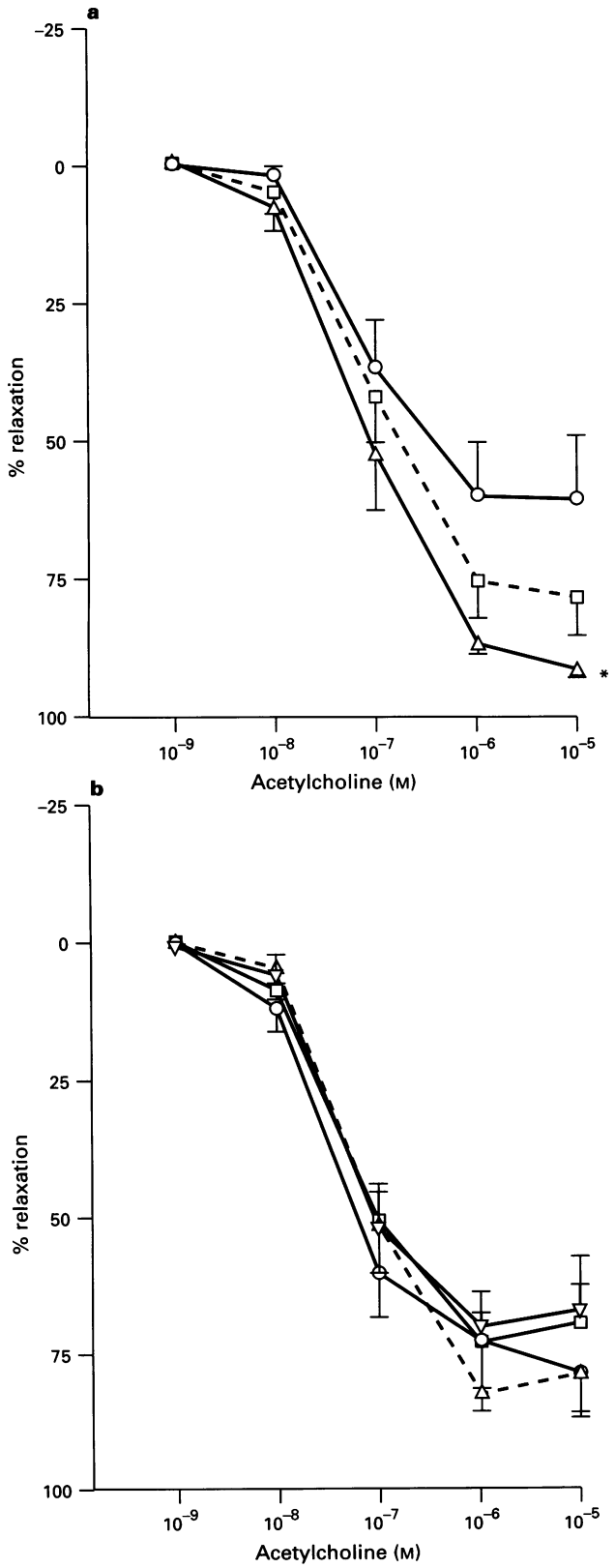

Figure 4 Acetylcholine-induced relaxation response of rat mesenteric resistance vessels from controls (○); insulin-treated diabetic rats (□); untreated diabetic rats (△) and islet transplanted rats (▽-12 weeks only) in (a) 6 week study and (b) 12 week study. Points show means±s.e.mean.

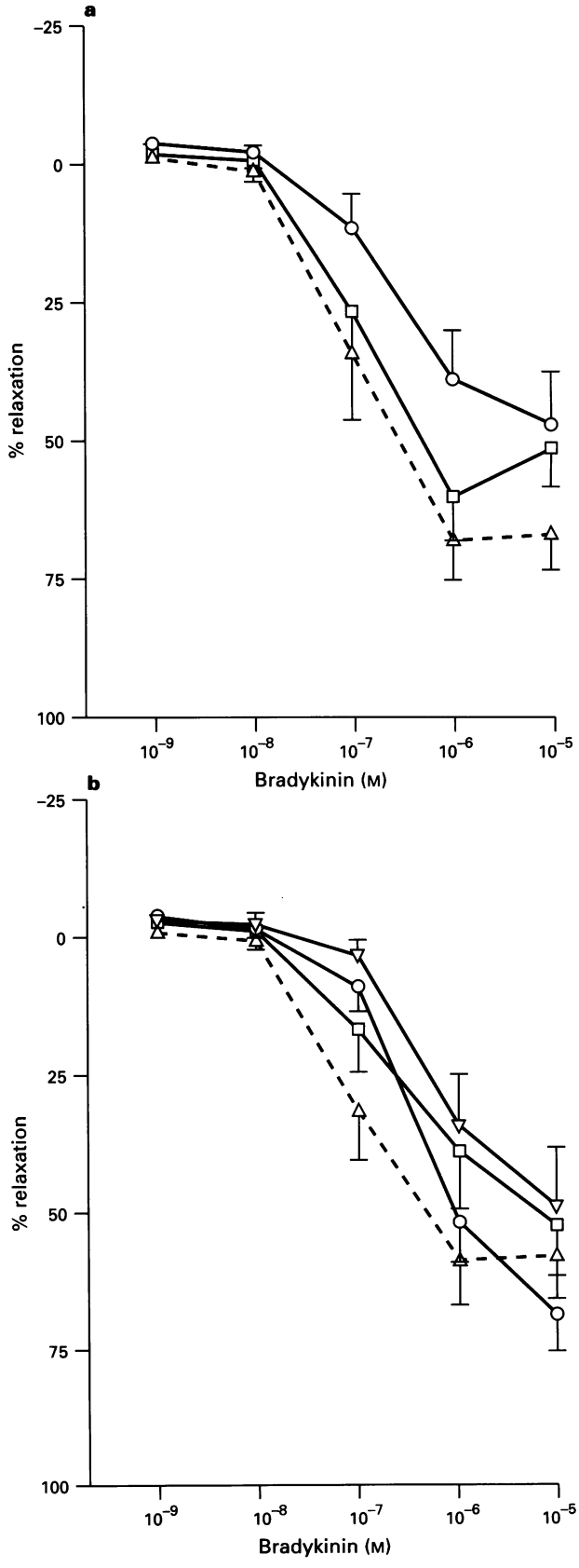

Figure 5 Bradykinin-induced relaxation response of rat mesenteric resistance vessels from controls (○); insulin-treated diabetic rats (□); untreated diabetic rats (△) and islet transplanted rats (▽-12 weeks only) in (a) 6 week study and (b) 12 week study. Points show means±s.e.mean.

betic: 7.15±0.11; transplants: 7.21±0.16). Incubation with L-NOARG caused significant attenuation in the maximum relaxation responses in all groups. However, in keeping with the six week study, there was no significant change in sensitivity following the addition of L-NOARG (Table 4).

Relaxation responses to bradykinin

Six week study There was no significant difference in maximum relaxation response in the diabetic animals compared to their age-matched controls (control: 54±8%, $n=8$; untreated diabetics: 71±6%, $n=7$; insulin-treated diabetics: 63±8%, $n=8$), although there was a significant shift in sensitivity between the insulin-treated diabetics and the controls (6.98±0.12 versus 6.48±0.12) (Figure 5a). A 1 h incubation with L-NOARG, significantly inhibited the BK relaxation response in all three groups. There was also a shift in sensitivity evident in both the insulin-treated ($P=0.059$), and the untreated diabetics ($P=0.052$), although statistical significance was not quite reached (Table 5).

Twelve week study There was no significant difference between maximum BK relaxation response in the experimental groups compared to the sham-operated non-diabetic controls (untreated: 63±7%, $n=8$; insulin-treated: 56±9%, $n=8$; and transplants: 49±11, $n=7$ versus controls: 68±7, $n=8$). Also there was no change in the sensitivity between the experimental groups and the non-diabetic controls (controls: 6.34±0.07; untreated diabetics: 6.85±0.18; insulin-treated diabetics: 6.44±0.15; transplants: 6.37±0.16) (Figure 5b). Incubation with the nitric oxide synthase inhibitor, L-NOARG, caused significant attenuation of the maximum relaxation response to bradykinin in all of the experimental groups, but there was no change in BK sensitivity after the addition of L-NOARG (Table 5).

Relaxation responses to sodium nitroprusside

Six week study There was not significant difference in the maximum relaxation response or sensitivity to the endothelium-independent vasodilator, sodium nitroprusside between the diabetic groups and the age-matched controls (controls: 84±3%, 6.29±0.07, $n=9$; untreated diabetics: 87±3%, 6.31±0.09, $n=8$; insulin-treated diabetics: 76±5%, 6.15±0.1, $n=8$), or when the untreated rats were compared directly to the insulin treated rats (Figure 6a).

Twelve week study The maximum relaxation and sensitivity to sodium nitroprusside was similar in all of the experimental groups (controls: 86±3%, 6.43±0.1, $n=8$; untreated diabetics: 84±4%, 6.33±0.27, $n=8$; insulin-treated diabetics: 90±4%, 6.69±0.12, $n=7$; transplants: 87±4%, 6.5±0.18, $n=8$; Figure 6b).

Discussion

The results of this study support increased basal and stimulated release of nitric oxide in streptozotocin-induced diabetic rats at both six and twelve weeks duration. Thus incubation with the nitric oxide synthase inhibitor, N^G-nitro-L-arginine (L-NOARG), revealed a significant increase in contractility and sensitivity in the untreated diabetic vessels at six weeks, with increased sensitivity also displayed at 12-weeks. Insulin-treated diabetic rats showed increased sensitivity at both 6 and 12-weeks, with the 12-week insulin-treated diabetics displaying enhanced contractility to noradrenaline. However, when the ratio of shift in sensitivity to noradrenaline (after incubation with L-NOARG) was calculated, and then compared between each of the experimental groups, no significant enhancement of sensitivity to noradrenaline was evident in either the 6 or the 12-week study. Vascular reactivity to noradrenaline in the transplanted group remained unaltered, and was not significantly different to the controls. This is in agreement with the only other transplant study which has directly investigated the responses of rat blood vessels to contractile and relaxant agents (Pieper *et al.*, 1995).

In contrast to these findings, some groups who investigated aortic responses to noradrenaline reported that there was no significant difference in contractility in the diabetic vessels (Cameron & Cotter, 1992), especially when contractile force was related to tissue weight (Oyama *et al.*, 1986). However, other studies of rats with chemically induced diabetes have demonstrated an increased contractile response to noradrena-

Table 4 Endothelium-dependent relaxation and sensitivity to acetylcholine before and after incubation with N^G-nitro-L-arginine (L-NOARG) in six and twelve week STZ study

	ACh Max.	*+L-NOARG*	*pD_2*	*+L-NOARG*
6-week Control ($n=8$)	64±10%*	30±9%	6.0±0.11	7.1±0.25
6-week Diabetic ($n=7$)	92±1%*	71±6%	6.96±0.1	6.66±0.11
6-week Diabetic+insulin ($n=8$)	79±7%*	35±11%	6.99±0.08	7.01±0.07
12-week Control ($n=10$)	86±3%*	34±8%	7.1±0.07	7.01±0.13
12-week Diabetic ($n=8$)	84±3%*	48±9%	7.1±0.1	7.03±0.2
12-week Diabetic+insulin ($n=8$)	75±5%*	48±8%	7.15±0.11	7.09±0.1
Transplants ($n=7$)	72±7%*	39±11%	7.21±0.16	6.99±0.15

*$P<0.05$ (paired t test before and after L-NOARG); †$P<0.05$ (unpaired t test, untreated diabetic versus control).

Table 5 Endothelium-dependent relaxation and sensitivity to bradykinin before and after incubation with N^G-nitro-L-arginine (L-NOARG) in six and twelve weeks STZ study

	BK Max.	*+L-NOARG*	*pD_2*	*+L-NOARG*
6-week Control ($n=8$)	54±8%**	18±5%	6.48±0.12	6.42±0.14
6-week Diabetic ($n=7$)	71±6%*	49±4%	6.77±0.13	6.39±0.1
6-week Diabetic+insulin ($n=8$)	63±8%*	28±11%	6.98±0.12†	6.42±0.07
12-week Control ($n=8$)	68±7%**	20±7%	6.34±0.07	5.92±0.11
12-week Diabetic ($n=8$)	63±7%**	25±7%	6.85±0.18	6.72±0.15
12-week Diabetic+insulin ($n=8$)	56±9%*	27±10%	6.44±0.15	6.45±0.14
Transplants ($n=7$)	49±11%*	19±7%	6.37±0.16	6.53±0.34

*$P<0.05$, **$P<0.01$ (paired t test before and after L-NOARG); †$P<0.05$ (diabetic+insulin versus control).

line in aortae (Mulhern & Docherty, 1989), mesenteric arteries (Agrawal & McNeill, 1987; White & Carrier, 1988), isolated perfused hindquarters (Friedman, 1989), and perfused kidneys (Bhardwaj & Moore, 1988). Another investigation of vascular reactivity of both aortae and mesenteric arteries in chronic experimental diabetes showed that the increased contractile response to noradrenaline was evident only in the untreated diabetics, whereas treatment with insulin completely prevented the increase (Macleod, 1985). Similar findings were reported in a long-term study of diabetes where the aortae from untreated diabetic rats showed an increased sensitivity to the agonist, phenylephrine, which was partially corrected in the insulin-treated group (Chang & Stevens, 1992).

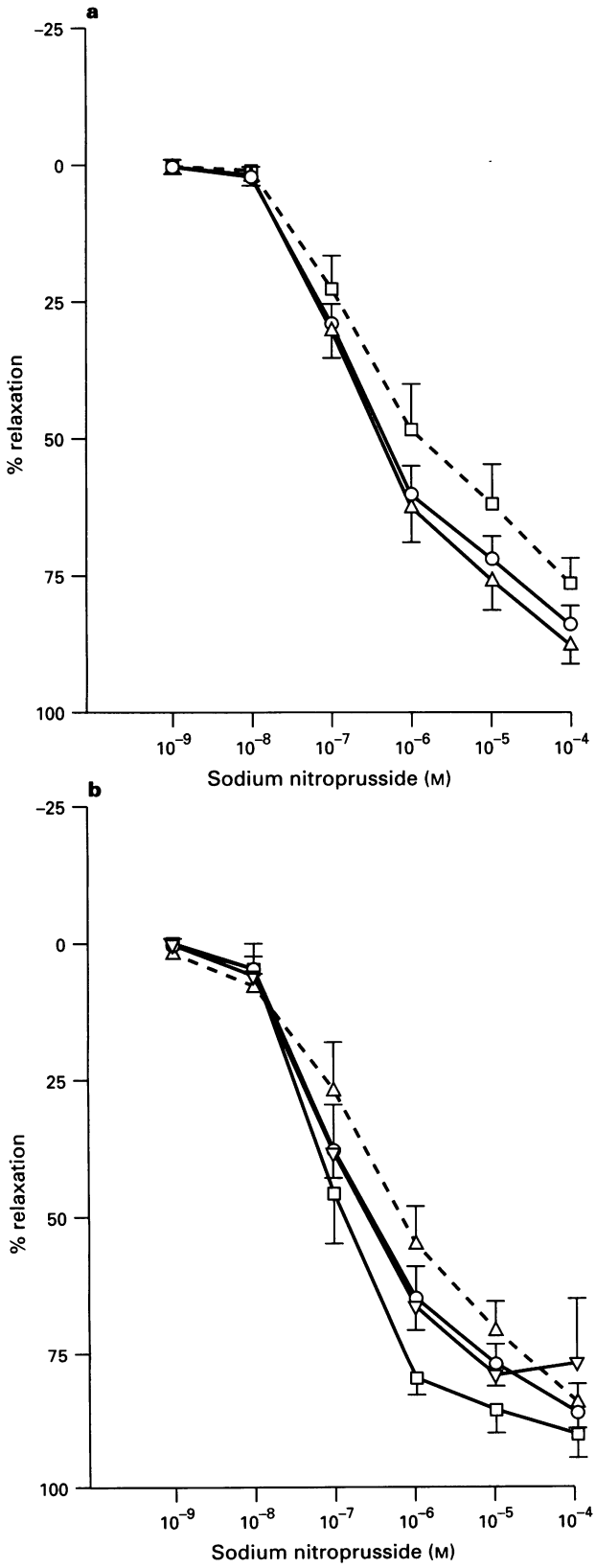

Figure 6 Endothelium-induced relaxation response to sodium nitroprusside of rat mesenteric resistance vessels from controls (○); insulin-treated diabetic rats (□); untreated diabetic rats (△) and islet-transplanted rats (▽-12 weeks only) in (a) 6 week study and (b) 12 week study. Points show means ± s.e.mean.

In one study of resistance artery function, of similar experimental design to our study, evidence of enhanced contractility to noradrenaline was revealed in both untreated and insulin treated diabetic rats, and similarly, incubation with a nitric oxide synthase inhibitor (L-NAME) resulted in an increase in sensitivity to noradrenaline in the diabetic vessels (Taylor *et al.*, 1994a). Likewise, the maximum contractile response to depolarizing K^+PSS also was significantly increased in the insulin-treated diabetic group.

Interestingly, in the activation procedure of the 12-week study, contractile responses to the depolarizing K^+PSS, and NAK^+ were significantly augmented in the insulin-treated diabetic group alone, suggesting a chronic effect of raised exogenous insulin on the contractile mechanism of the vascular smooth muscle. In this study, the insulin-treated rats received their last injection 24 h previously and so plasma insulin levels in the freshly culled rat should be negligible. It is however, possible that the insulin-treated rats were hyperinsulinaemic and that this effect was carried over into the myograph bath. Indeed, other workers have also demonstrated a similar effect after *in vivo* treatment of diabetic rats with insulin (Taylor *et al.*, 1994a).

There was no evidence of impaired endothelium-dependent relaxation to acetylcholine or bradykinin in either the untreated or the insulin-treated diabetics of the six or twelve week study; this is in agreement with other studies which investigated the vasodilator response of the mesenteric vascular bed (Kiff *et al.*, 1991a), and the aortae (Head *et al.*, 1987; Taylor *et al.*, 1994b) or chemically-induced diabetic rats. In our 6-week study, maximum relaxation to ACh was significantly *augmented* in the untreated diabetic group. This increased vasodilator response again suggests an increased release of nitric oxide, which appears to be absent in more established diabetes of longer duration (i.e. 12–14 weeks). Indeed, further evidence to support this is again provided by the incubation of the vessels with L-NOARG. In the presence of the nitric oxide synthase inhibitor, the untreated diabetic vessels from the 6-week study failed to display such a marked reduction in endothelium-dependent relaxation as that of the insulin-treated diabetic and control vessels.

An alternative explanation for the increased relaxation response to ACh is the release of an additional relaxing factor, namely endothelium-derived hyperpolarizing factor (EDHF). This has been shown to relax vascular smooth muscle cells by opening potassium channels, and hyperpolarizing the cell membrane (Van de Voorde *et al.*, 1992). It is possible that the simultaneous release of vasodilator eicosanoids could also be responsible for altering endothelium-mediated relaxation to acetylcholine in the diabetic blood vessels. Several studies have demonstrated augmented release of prostacyclin from diabetic arteries, and this increased production, coupled with its synergistic action on EDRF may play an important role in mediating endothelium-dependent relaxation in certain diabetic vascular beds (White & Carrier, 1986).

In keeping with contractile agonists, the literature on the responses to endothelium-dependent vasodilators is consistent; with increased relaxation being reported in one study (White & Carrier, 1986), and a decrease in sensitivity, but not maximum relaxation, being reported in another (Kamata *et al.*, 1989). In contrast, other investigations of endothelial-dependent responses in chemical induced diabetes, have demonstrated *attenuation* of endothelium-dependent relaxation to ACh in both mesenteric arteries (Taylor *et al.*, 1994b) and aortae (Oyama *et*

al., 1986; Cameron & Cotter, 1992) from diabetic rats. Also, we have shown impaired endothelium-dependent relaxation in the resistance arteries from the spontaneously diabetic Bio Bred (BB) rat (Heygate et al., 1995).

However, acetylcholine is not an important physiological mediator of endothelium-dependent relaxation *in vivo*, but the influence of shear stress on the subsequent release of NO has been directly linked to the same G-protein as ACh (Ohno et al., 1993). It is therefore possible to extrapolate from these findings, and suggest that flow-induced NO release could be abnormal in diabetes, and may have important implications in the determination of peripheral tone. Indeed, increased blood flow has been shown in the early stages of insulin-dependent diabetes mellitus in man, resulting in precapillary vasodilatation (Jaap & Tooke, 1995). One *in vivo* animal study which compared regional blood flow in different vascular beds in rats, which had been diabetic for four weeks, reported a significant increase in blood flow to the mesenteric bed, whereas, blood flow to other abdominal and thoracic organs was unchanged, and those to skin and muscle was reduced (Hill & Larkins, 1989). Moreover, increased renal and mesenteric blood flow has been demonstrated in conscious, chronically instrumented rats following streptozotocin treatment (Kiff et al., 1991a,b).

The relaxation response to the endothelium-independent vasodilator, sodium nitroprusside in this study, was similar in all the experimental groups irrespective of the duration of diabetes. The literature reveals conflicting reports about alterations in the SNP response in diabetes. The majority of studies show that the SNP response is not impaired in diabetics versus controls (Oyama et al., 1986; Pieper et al., 1995; Taylor et al., 1995), but one study showed a decrease in relaxation to SNP in diabetic arteries (Kiff et al., 1991a). Similarly, in man some studies in insulin-dependent diabetic subjects, have shown normal responses to SNP (Elliott et al., 1993; McNally et al., 1994), whilst another has demonstrated an impaired response (Calver et al., 1992).

The transient changes in reactivity observed in these diabetic animal and human studies, suggest that complex alterations in vascular function develop in early stages of diabetes, and that these changes continue to be modulated by other intrinsic or extrinsic factors that come into play as the disease progresses (Katz & McNeill, 1987; Kabbah et al., 1988). Since the arterioles are the major site of vascular resistance, small changes in arteriolar sensitivity could have a significant effect on blood pressure, and the regulation of tissue blood flow (Morff, 1990).

Several studies of islet transplantation in diabetic rats have been performed demonstrating the potential to reverse the diabetic state and associated microvascular complications (Mauer et al., 1975; Orloff et al., 1987; Schmidt 1983). One such study reported that the development of nerve fibre abnormalities could be prevented if islets were transplanted soon after the onset of diabetes. However, where islet transplantation was delayed, abnormal fibre morphology occurred, and complete reversal of the diabetic neuropathy was not achieved despite consistent euglycaemia (Britland et al., 1991).

More recently, a study has shown that islets transplanted either beneath the kidney capsule, or intraportally to the liver, and the subsequent long-term reversal of hyperglycaemia, can prevent the development of glomerular basement membrane thickening found in untreated diabetic animals (Leow et al., 1995). Interestingly, another recent study demonstrated that pancreatic islet transplantation not only restored euglycaemia, but also reversed the endothelial dysfunction in rat aortae (Pieper et al., 1995). Moreover, this group showed islet transplantation completely normalized indices of oxidative stress in various blood vessels even after eight weeks of diabetes (Pieper et al., 1995); and since oxidizing free radicals are known to have a detrimental effect on endothelial function, this improvement must be considered to be an additional benefit of islet transplantation.

In conclusion, we can suggest that in the early stages of diabetes there is an enhanced release of nitric oxide, which was more evident in the untreated diabetic rats, than the insulin-treated, and appeared to normalize as the duration of diabetes progressed. This study also uniquely showed that the alteration in vascular reactivity of the resistance arteries was restored within normal limits by the transplantation of islets of Langerhans. Islet transplantation also successfully corrected the metabolic abnormalities of the insulin-dependent diabetic state, and did not appear to have any detrimental effects on the contractile and relaxation responses of the mesenteric resistance vasculature. Further studies of islet transplantation in diabetic rats with different lengths of disease duration are necessary to determine whether an end-point of the reversibility of the diabetic state, the amelioration of altered vascular reactivity or indeed, endothelial dysfunction exists.

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