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Effects of diabetes and treatment with the antioxidant α-lipoic acid on endothelial and neurogenic responses of corpus cavernosum in rats

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Summary Diabetes mellitus is associated with impotence in animal models and patients. Raised reactive oxygen species contribute to diabetic neurovascular deficits, which are amenable to antioxidant treatment. Our aim was to examine the effects of streptozotocin-induced diabetes in rats and long-term treatment with the antioxidant, α-lipoic acid, on responses of an in vitro corpus cavernosum preparation. Diabetes duration was 8 weeks and preventive and reversal (4 weeks untreated diabetes, 4 weeks of treatment) studies were done. Four and 8 weeks of diabetes caused an about 41 % reduction in endothelium-dependent nitric oxide mediated relaxation to acetylcholine in phenylephrine-precontracted cavernosum. This deficit was prevented (93.9 ± 7.1 %) by treatment with α-lipoic acid; reversal studies showed 64.9 ± 19.5 % correction. Neither diabetes nor treatment with α-lipoic acid altered endothelium-independent relaxation to the nitric oxide donor, sodium nitroprusside. Stimulation of corpus cavernosum autonomic innervation caused noradrenergic-mediated contractions that were unaffected by diabetes or α-lipoic acid. Non-adrenergic, non-cholinergic nerve responses, largely dependent on nitric oxide, were seen after phenylephrine precontraction in the presence of atropine and guanethidine. Non-adrenergic, non-cholinergic stimulation caused frequency dependent relaxation to a maximum of about 40 %. Diabetes reduced this to about 25 %, however with preventive α-lipoic acid treatment, non-adrenergic, non-cholinergic relaxation was in the nondiabetic range. In the reversal α-lipoic acid treated diabetic group, its deficit was corrected by 52.1 ± 14.6 %. Thus, diabetes reduces endothelium and non-adrenergic, non-cholinergic nerve nitric oxide-mediated relaxation of corpus cavernosum smooth muscle, which is likely to be the organic base for impotence. Prevention and partial correction by α-lipoic acid emphasises the importance of reactive oxygen species and suggests a potential therapeutic approach. [Diabetologia (1999) 42: 343–350]

Keywords Diabetes mellitus, corpus cavernosum, autonomic neuropathy, antioxidant, nitric oxide, impotence, rat.

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Abbreviations: ACh, Acetylcholine; AGE, advanced glycation end product; GSH, reduced glutathione; LA, α-lipoic acid; NO, nitric oxide; NOS, nitric oxide synthase; NANC, non-adrenergic non-cholinergic; ROS, reactive oxygen species; SNP, sodium nitroprusside; TEFS, transmural electrical field stimulation; VIP, vasoactive intestinal polypeptide.

Males with diabetes mellitus have an increased prevalence of erectile dysfunction. Some of the organic causes have been studied in corpus cavernosum strips isolated from men undergoing penile prosthesis implantation. Defects in endothelium-dependent and nerve-mediated vascular smooth muscle relaxation have been found [1]. Similar deficits were noted for alloxan-diabetic rabbits [2] and recently diminished neurogenic erections were reported in streptozotocin-diabetic rats [3]. The nitric oxide (NO) systems of corpus cavernosum endothelium and nerve supply are crucial for smooth muscle relaxation and erectile

function [4, 5] and in some diabetic rat models cavernosal NO synthase (NOS) is diminished [6].

Impaired NO-mediated vasodilation is commonly found in vivo and in vitro for individual vessels and vascular beds of diabetic animal models [7–17] and has been noted in several studies on patients [18–21]. One potential cause is the raised level of reactive oxygen species (ROS) in diabetes [22], which could react with NO to neutralise its vasodilator activity [23]. Defects in endothelium-dependent relaxation of large and small vessels could be prevented by antioxidant treatment in diabetic rats [9–12], although there has been a recent dissenting report for mesenteric vessels [24]. α-Lipoic acid (LA) is a powerful antioxidant, possessing both radical scavenger and transition metal chelator properties [25], that has recently been shown to improve nerve blood flow, glutathione (GSH) content and motor and sensory conduction velocity in diabetic rats [26, 27]. The aims of this investigation were to assess the effects of diabetes and long-term LA treatment on endothelial and neurogenic function using an in vitro rat corpus cavernosum preparation [28].

Materials and methods

Male Sprague-Dawley rats (Aberdeen University colony) were used, aged 19 weeks at the start of experiments. Diabetes was induced by intraperitoneal injection of streptozotocin (Zeneca, Macclesfield, Cheshire, UK) freshly made up in sterile 154 mmol · l^{-1} NaCl solution, at a dose of 40–45 mg · kg^{-1}. Diabetes was verified after 24 h by the presence of hyperglycaemia and glucosuria (Visidex II and Diastix; Ames, Slough, UK) in non-fasted rats. After that, rats were tested weekly, and weighed daily; they were rejected if blood glucose was < 20 mmol · l^{-1} or if they showed a consistent increase in body weight over 3 days.

After final experiments, plasma glucose was estimated (GOD-Perid method; Boehringer Mannheim, Mannheim, Germany) on samples taken from the tail vein. Diabetes duration was 8 weeks and two studies, prevention and reversal, were undertaken. In the preventive study, one group acted as diabetic controls and another group was treated with LA as a dietary supplement (dose 300 mg · kg^{-1} · day^{-1} racemate; ASTA Medica AWD, Frankfurt, Germany). Treatment was started within 2 days of streptozotocin injection once the hyperglycaemic state was confirmed. Onset and age-matched groups were used as untreated nondiabetic controls and a further group of nondiabetic rats was given LA treatment (300 mg · kg^{-1} · day^{-1} racemate). In the reversal study, groups comprised onset nondiabetic controls, 4-week and 8-week diabetic controls and a group of diabetic rats untreated for the first 4 weeks and then given LA treatment (300 mg · kg^{-1} · day^{-1} racemate) for the last 4 weeks.

In final experiments, rats were anaesthetised (4% halothane in air), the penis was excised at its base and the shaft was dissected free of connective and adventitial tissue. The copora cavernosa were separated, a longitudinal slit was made in each cavernosum to aid drug penetration and they were mounted separately in organ baths. Cavernosa were bathed in modified Krebs-Ringers solution (144.0 Na^+, 5.0 K^+, 1.25 Ca^{2+}, 1.1 Mg^{2+}, 25.0 HCO_3^-, 1.1 PO_4^{3-}, 1.1 SO_4^{2-}, 5.5 glucose; in mmol · l^{-1}) at 37 °C and gassed continuously with 95% O_2:5% CO_2 (pH 7.35). Tension was monitored by isometric transducers and resting tension was set at 1.5 *g*, which was established in pilot experiments as optimal for tension production when cavernosa were stimulated by phenylephrine [28]. Tissues were equilibrated for 60 min, then they were precontracted with phenylephrine at a dose (30 μmol · l^{-1}) giving about 60% of the maximum contraction in all groups [28]. Cumulative concentration-response relations were determined for endothelium-dependent relaxation to acetylcholine (ACh) and endothelium-independent relaxation to sodium nitroprusside.

Repetitive supramaximum transmural electrical field stimulation (TEFS) of autonomic nerves was accomplished via platinum wire electrodes placed either side of the cavernosum (duration 15 s; amplitude 50–90 V; frequency range 2–20 Hz; pulse width 5 ms). Frequency-response curves were determined for noradrenergic vasoconstrictor nerves. Preparations were then preincubated for 30 min with guanethidine (5 μmol · l^{-1}) to eliminate responses mediated by noradrenergic nerves, and atropine (1 μmol · l^{-1}) to prevent cholinergic nerve responses. After phenylephrine (30 μmol · l^{-1}) precontraction under these conditions, TEFS showed relaxation mediated by non-adrenergic, non-cholinergic (NANC) fibres. In preliminary experiments on cavernosa from nondiabetic ($n = 4$), diabetic ($n = 4$) and prevention LA-treated diabetic ($n = 4$) rats, the specificity of responses to neural stimulation was tested by preincubation with tetrodotoxin (1 μmol · l^{-1}) for 30 min.

Statistical analysis. Data are expressed as means ± SEM. They were subjected to Bartlett's test for homogeneity of variances and where necessary they were normalised by log transformation before being tested using one-way analysis of variance. When significance was attained ($p < 0.05$), between-group differences were established using the Student-Newman-Keuls multiple comparison test. Concentration-response curves were fitted by sigmoid curves using the least squares method to estimate EC_{50}. Calculations were made using a standard statistical software package (Prism, Graphpad, San Diego, Calif., USA).

Results

Diabetic groups had an approximate fourfold increase of plasma glucose concentration (Table 1), which was not significantly altered by LA treatment in prevention or reversal groups. This treatment also did not affect plasma glucose in nondiabetic rats compared with onset or age-matched controls. Diabetic rats showed about 30% weight loss over the experimental period, which was not significantly affected by LA treatment. In contrast, nondiabetic rats increased their weight by about 7% over the 8-week experimental period, except for the LA-treated nondiabetic group, where there was a small weight loss (6.2%; $p < 0.001$, paired Student's *t*-test).

Preventive study. Endothelium dependent relaxation (Fig. 1) to ACh was impaired by diabetes ($p < 0.001$), maximum relaxation being 29.4 ± 3.0% compared to onset (51.2 ± 2.5%) or age-matched (47.0 ± 1.5%)

Table 1. Body weights and plasma glucose concentrations for rat groups in prevention and reversal studies

Group	*n*	Body weight (g)		Plasma glucose (mmol · l^{-1})
		Start	End	
Nondiabetic				
Onset control	30	427 ± 4	–	10.1 ± 0.6
Age-matched control	9	419 ± 5	451 ± 6	9.5 ± 0.8
α-Lipoic acid treatment	9	437 ± 4	410 ± 4	11.8 ± 1.2
Diabetic				
4 weeks	11	451 ± 10	345 ± 9	43.8 ± 1.3
8 weeks	27	449 ± 4	293 ± 5	43.8 ± 1.3
α-Lipoic acid prevention treatment	15	450 ± 7	301 ± 6	39.0 ± 2.7
α-Lipoic acid reversal treatment	11	444 ± 5	302 ± 5	41.7 ± 3.1

Data are means ± SEM

Table 2. Effects of 4-h preincubation in 5.5 or 40 mmol · l^{-1} glucose on relaxation to acetylcholine of phenylephrine-precontracted corpus cavernosa from nondiabetic and diabetic rats

Group	*n*	Precontraction tension (g · g wet wt^{-1})	Maximum relaxation (%)	(-log) EC_{50} (mol · l^{-1})
Nondiabetic	8			
5.5 mmol · l^{-1} glucose		2.28 ± 0.42	46.2 ± 2.6	5.76 ± 0.21
40 mmol · l^{-1} glucose		2.62 ± 0.48	43.3 ± 1.8	5.81 ± 0.14
8 week diabetic	6			
5.5 mmol · l^{-1} glucose		2.39 ± 0.25	24.5 ± 2.9[a]	6.00 ± 0.17
40 mmol · l^{-1} glucose		2.51 ± 0.41	26.1 ± 2.9[a]	5.81 ± 0.14

Data are means ± SEM. [a] $p < 0.001$ vs nondiabetic group exposed to the same glucose concentration

Table 3. Effects of diabetes and α-lipoic acid treatment on corpus cavernosum relaxation to sodium nitroprusside

Group	*n*	Maximum relaxation (%)	(-log) EC_{50} (mol · l^{-1})
Nondiabetic control	9	48.8 ± 2.5	5.88 ± 0.17
Nondiabetic + α-lipoic acid	9	50.9 ± 4.1	5.76 ± 0.14
Diabetic control	9	47.6 ± 3.4	5.76 ± 0.20
Diabetic + α-lipoic acid	9	47.7 ± 2.5	6.24 ± 0.19

Data are means ± SEM

control values. Treatment with LA did not alter maximum relaxation (51.5 ± 2.9 %) in nondiabetic rats. In strong contrast, maximum relaxation was in the nondiabetic range (47.9 ± 1.4 %) for LA-treated diabetic rats ($p < 0.001$ vs diabetic control group). Neither diabetes nor LA treatment altered sensitivity to ACh; (-log)EC_{50} values were 5.86 ± 0.13, 5.74 ± 0.10, 5.65 ± 0.20, 5.69 ± 0.12 and 5.77 ± 0.10 for onset and age-matched control, LA-treated nondiabetic, diabetic control and LA-treated diabetic groups, respectively.

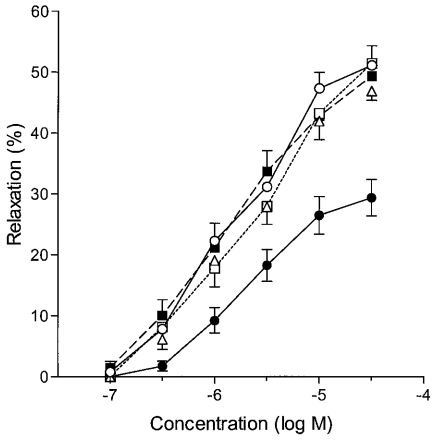

Fig. 1. Concentration-response curves for relaxation to acetylcholine after phenylephrine precontraction of corpus cavernosa from nondiabetic and diabetic rats in vitro and the effects of chronic preventive α-lipoic acid treatment. Groups: nondiabetic onset control (○, solid line, $n = 14$); nondiabetic age-matched control (Δ, $n = 9$); nondiabetic group treated with 300 mg · kg^{-1} · day^{-1} α-lipoic acid for 8 weeks (□, fine dashed line, $n = 9$); 8-week diabetic control (●, solid line, $n = 14$); 8-week diabetic rats treated from induction with 300 mg · kg^{-1} · day^{-1} α-lipoic acid (■, coarse dashed line, $n = 15$). Data are means ± SEM. Statistics: $p < 0.05$, diabetic control group vs onset control and lipoic acid treated diabetic groups for acetylcholine concentrations ≥ 1 μmol · l^{-1}

The reduced responses to ACh could not be mimicked in corpus cavernosa from nondiabetic rats perifused with a solution high in glucose. Thus, paired experiments (Table 2) were carried out in which one cavernosum from a rat was preincubated for 4 h in 5.5 mmol · l^{-1} glucose and the other one was exposed to 40 mmol · l^{-1} glucose for the same time period. There were no significant differences in the tension generated in response to the 30 μmol · l^{-1} phenylephrine precontracting stimulus and there were no significant changes in maximum relaxation or (-log)EC_{50} to ACh. Moreover, the diabetic deficit in ACh relaxation was unaffected by ambient glucose concentration at the time of measurement (Table 1). Acetylcholine-induced relaxation responses at 5.5 mmol · l^{-1} glucose were unaffected by preincubation with the cyclooxygenase inhibitor, flurbiprofen (10 μmol · l^{-1}) but were completely abolished by NOS inhibition with 10 μmol · l^{-1} N^G-nitro-L-arginine (data not shown).

In contrast to the findings for acetylcholine, endothelium-independent relaxation to sodium nitroprusside (SNP) in phenylephrine-precontracted corpora cavernosa was unaffected by diabetes or LA treatment for maximum relaxation or (-log)EC_{50} (Table 3).

Transmural electrical field stimulation of corpus cavernosum caused frequency-dependent contractions (Fig. 2) that were completely blocked by guanethidine (5 μmol · l^{-1}) or tetrodotoxin

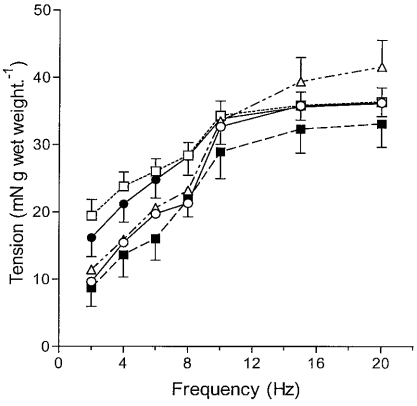

Fig. 2. Frequency-response curves for tension production in response to transmural electrical field stimulation of corpus cavernosa from nondiabetic and diabetic rats and the effects of long-term preventative α-lipoic acid treatment. Groups: nondiabetic onset control (○, solid line, $n = 15$); nondiabetic age-matched control (Δ, dot-dashed line, $n = 9$); nondiabetic group treated with 300 mg · kg^{-1} · day^{-1} α-lipoic acid for 8 weeks (□, fine dashed line, $n = 9$); 8-week diabetic control (●, solid line, $n = 15$); 8-week diabetic rats treated from induction with 300 mg · kg^{-1} · day^{-1} α-lipoic acid (■, coarse dashed line, $n = 15$). There were no statistically significant between-group differences at any stimulation frequency

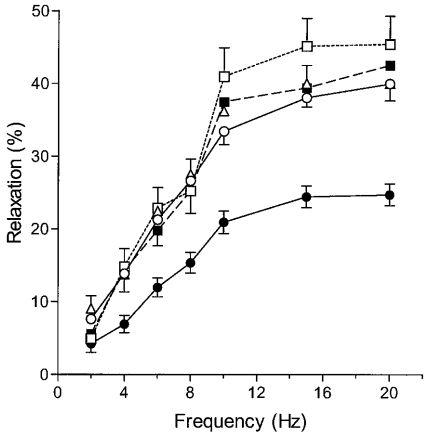

Fig. 3. Frequency-response curves for relaxation in response to transmural electrical field stimulation of corpus cavernosa from nondiabetic and diabetic rats in the presence of guanethidine and atropine, and the effects of long-term preventative α-lipoic acid treatment. Groups: nondiabetic onset control (○, solid line, $n = 18$); nondiabetic age-matched control (Δ, dot-dashed line, $n = 9$); nondiabetic group treated with 300 mg · kg^{-1} · day^{-1} α-lipoic acid for 8 weeks (□, fine dashed line, $n = 10$); 8 week diabetic control (●, solid line, $n = 18$); 8 week diabetic rats treated from induction with 300 mg · kg^{-1} · day^{-1} α-lipoic acid (■, coarse dashed line, $n = 18$). Data are means ± SEM. Statistics: $p < 0.05$, diabetic control group vs all other groups at 6–20 Hz

(1 μmol · l^{-1}). When expressed in terms of tension production per tissue weight, contractions were not significantly altered by diabetes or LA treatment. For cavernosa precontracted with phenylephrine, in the presence of guanethidine to block the noradrenergic contractile responses, and N^G-nitro-L-arginine (10 μmol · l^{-1}) to block nitrergic relaxation responses, a minor relaxation to TEFS was observed in nondiabetic tissues (maximum at 20 Hz; 7.8 ± 1.3 %, $n = 6$). This was unaffected by 8 weeks of diabetes (7.9 ± 1.5 %, $n = 6$) and was abolished by atropine (1 μmol · l^{-1}), suggesting that this relaxation was derived from cholinergic muscarinic fibres.

Transmural electrical field stimulation in phenylephrine precontracted cavernosa, pretreated with both guanethidine and atropine, gave frequency-dependent relaxation that was completely abolished by N^G-nitro-L-arginine (10 μmol · l^{-1}) or tetrodotoxin (1 μmol · l^{-1}). This NANC relaxation (Fig. 3) reached a maximum of 40.0 ± 1.5 % and 40.1 ± 2.4 % in onset and age-matched control groups, respectively. Diabetes blunted the response by 38 % to 24.7 ± 1.5 % ($p < 0.001$). Treatment with LA did not significantly affect NANC relaxation in cavernosa from nondiabetic rats (45.5 ± 3.9 %), however, in diabetic rats, relaxation (42.6 ± 2.6 %) was in the nondiabetic range, greater ($p < 0.001$) than for the diabetic control group.

The NANC responses were not significantly influenced by short-term exposure to glucose. In paired experiments where one cavernosum from a rat was preincubated for 4 h in 5.5 mmol · l^{-1} glucose and the other one was exposed to 40 mmol · l^{-1} glucose for the same period, maximum relaxation at 20 Hz in nondiabetic rats ($n = 6$) was 37.4 ± 2.3 % at 5.5 mmol · l^{-1} glucose and 35.0 ± 3.4 % at 40 mmol · l^{-1} glucose. In tissues from diabetic rats ($n = 5$), maximum relaxation was 27.2 ± 2.8 % at 5.5 mmol · l^{-1} and 28.5 ± 2.6 % at 40 mmol · l^{-1}.

Reversal study. Relaxation to ACh in phenylephrine-precontracted corpus cavernosa is shown in Figure 4. Compared with maximum relaxation for nondiabetic onset controls (48.9 ± 2.1 %), there were about 40 % deficits with diabetes of 4 (28.4 ± 4.9 %; $p < 0.01$) or 8 (29.8 ± 2.9 %; $p < 0.001$) weeks duration. Treating diabetic rats with LA during the last 4 weeks caused an about 65 % reversal of the diabetic deficit such that maximum relaxation (41.7 ± 4.0 %) was improved compared with 4 ($p < 0.05$) and 8 ($p < 0.05$) week diabetic groups and was in the lower half of the nondiabetic range. There were no significant between-group differences in ACh (-log)EC_{50} values which were 5.64 ± 0.15, 5.49 ± 0.12, 5.66 ± 0.13 and 5.71 ± 0.09 for onset control, 4- and 8-week diabetic control and reversal LA treated diabetic groups respectively. There were no significant between-group differences in endothelium-independent relaxation to SNP for maximum relaxation or (-log)EC_{50} (data not shown).

The NANC responses to TEFS in phenylephrine-precontracted cavernosa preincubated with guanethi-

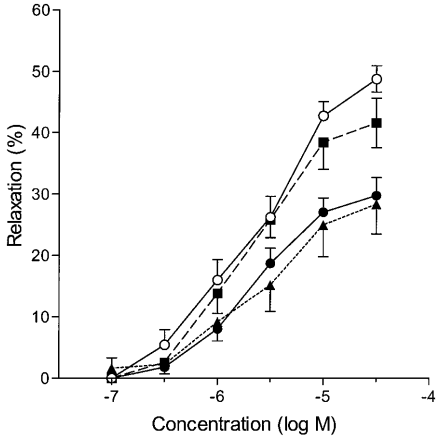

Fig. 4. Concentration-response curves for relaxation to acetylcholine following phenylephrine precontraction of corpus cavernosa from nondiabetic and diabetic rats and the effects of chronic reversal α-lipoic acid treatment. Groups: nondiabetic onset control (○, solid line, $n = 12$); 4-week diabetic control (▲, fine dashed line, $n = 9$); 8-week diabetic control (●, solid line, $n = 12$); 8-week diabetic group untreated for 4 weeks and then treated with 300 mg · kg^{-1} · day^{-1} α-lipoic acid for the last 4 weeks (■, coarse dashed line, $n = 11$). Data are means ± SEM. Statistics: $p < 0.05$, 4- and 8-week diabetic control groups vs all other groups for acetylcholine concentrations ≥ 10 μmol l^{-1}

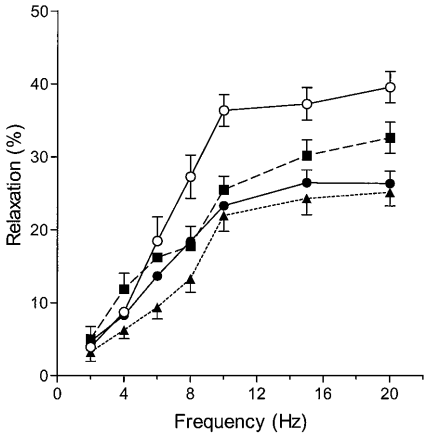

Fig. 5. Frequency-response curves for relaxation in response to transmural electrical field stimulation of corpus cavernosa from nondiabetic and diabetic rats and the effects of long-term reversal α-lipoic acid treatment. Groups: nondiabetic onset control (○, solid line, $n = 10$); 4-week diabetic control (▲, fine dashed line, $n = 11$); 8-week diabetic control (●, solid line, $n = 10$); 8-week diabetic group untreated for 4 weeks and then treated with 300 mg · kg^{-1} · day^{-1} α-lipoic acid for the last 4 weeks (■, coarse dashed line, $n = 10$). Data are means ± SEM. Statistics: $p < 0.05$, nondiabetic onset control group vs 4 week diabetic group 6–20 Hz, and 8-week diabetic group and α-lipoic acid treated diabetic group 8–20 Hz.; α-lipoic acid treated diabetic group vs 4-week diabetic group 15–20 Hz and 8-week diabetic group 20 Hz

dine and atropine were similarly attenuated by 4 and 8 weeks of diabetes (Fig. 5). Thus, maximum relaxation at 20 Hz was reduced from 39.6 ± 2.2 % in cavernosa from onset controls to 25.2 ± 1.9 % ($p < 0.001$) after 4 and 26.4 ± 1.7 % ($p < 0.001$) after 8 weeks of diabetes. Treating diabetic rats with LA during the second 4 week period caused about 52 % correction of the deficit such that maximum NANC relaxation was 32.7 ± 2.1 %, which was greater ($p < 0.05$) than 4- or 8-week diabetic controls, although an impairment remained ($p < 0.05$) compared with the control group.

Discussion

Diabetes caused deficits in endothelium-dependent and NANC nerve-mediated relaxation of corpus cavernosum smooth muscle, independent of the ambient glucose concentration during measurements. This agrees with previous reports in rat, rabbit and man [1–3]. Nitric oxide is the major vasodilator released by corpus cavernosum endothelium and a diabetic deficit in synthesis, release or action, is in line with many observations including those from in vitro studies on large arteries [7–9, 11, 12], small muscle arteries [15], heart and mesenteric vascular beds [10, 29] and in vivo studies on sciatic vasa nervorum [16, 17], brain [14, 30] and skin [21]. In the majority of these tissues, relaxation to NO donors, such as sodium nitroprusside or glyceryl trinitrate, was not affected by diabetes, as observed in this study for corpus cavernosum. In terms of an explanation for the ACh stimulated deficit, this rules out a defect in the cGMP mechanism and smooth muscle responsiveness to NO. Several possibilities remain which cannot be ruled out by our experimental findings, including abnormalities of ACh receptors or transduction mechanisms, reduced NO synthesis, and increased NO destruction.

A selective impairment of ACh transduction has been postulated for mesenteric vessels [31], however, at best this could only partially explain the corpus cavernosum data and is unlikely to apply more generally. Thus, NANC nerve relaxation, impaired by diabetes, is NO-mediated but ACh is not involved. Furthermore, in aorta, defective NO-mediated endothelium-dependent relaxation was noted when receptor-transduction systems were bypassed using a calcium ionophore [32].

Diminished NO synthesis could result from a reduction in NOS content or activity, or substrate depletion. The latter is unlikely since, like aorta, corpus cavernosum remains responsive to ACh over several serial cumulative dose-response determinations without decrement [11]. Furthermore, there is a greater dietary intake of L-arginine in diabetic rats and L-arginine transport is enhanced by diabetes/hyperglycaemia in human umbilical vein endothelial cells from women with gestational diabetes [33] although it is not known whether this effect is applicable to

other endothelial cells or chronic diabetes in rats. Reduced penis total (endothelial and neuronal) NOS activity and neuronal NOS levels have been reported after 4–8 months in BB/WOR and BBZ/WPR rat models of Type I (insulin-dependent) and Type II (non-insulin-dependent) diabetes mellitus [6]. In streptozotocin-diabetic rats of 2–3 months duration, increased penile endothelial and total NOS, however, was found using autoradiographic and biochemical and assays [34, 35]. It is possible that changes in NOS activity are biphasic in diabetic rats, an initial increase being followed by a decline. For the 4- and 8-week streptozotocin-diabetes durations used in this study, however, the literature suggests that at least endothelial NOS would be increased; therefore NOS changes would not explain the diminished relaxation to ACh, which was similar at both time points. It is possible that in the intact tissue other factors alter NOS responses. For example, advanced glycation and increased protein kinase C activation can reduce NOS activity [36–38]. Long-, but not short-term, treatment with aminoguanidine, which inhibits advanced glycation, prevents and reverses the endothelial NO defects that lead to reduced ACh responses and impaired tissue blood flow in diabetic rats [11, 13, 39]. Furthermore, protein kinase C inhibitors improve blood flow in retina and nerve, which depends on a NO mechanism [40, 41].

Even if corpus cavernosum NOS activity were normal in diabetic rats, by neutralising NO, the increased ROS in diabetes would reduce ACh-stimulated relaxation. Therefore, the prediction on this hypothesis is that ROS scavengers would improve endothelium-dependent relaxation. The antioxidant, LA, provided a high level of protection and partially reversed an established defect. This is the first report of successful treatment of impaired corpus cavernosum endothelial responses in experimental diabetes. Like vitamin E, LA is a scavenger however, it is a transition metal chelator, therefore it inhibits important sources of ROS formation in diabetes by metal catalysed processes including the Fenton reaction and advanced glycation [42]. This results in LA being about ten times more potent than vitamin E against vascular deficits in diabetic rats [27, 43]. The data are in general agreement with the literature on protective effects of scavenger and metal chelator treatment on endothelium dependent relaxation of blood vessels [9–12]. The observation that vitamin E does not preserve mesenteric vessel responses in diabetes is at odds with this conclusion, however, concomitant vitamin E exposure protected aorta, coronary circulation and vasa nervorum [9, 10, 43], which argues against the generality of that finding. Furthermore, mesenteric responses are protected by LA [44], showing that they are amenable to potent antioxidant treatment.

The finding that LA only partially reversed an existing endothelial deficit suggests that defective NO-mediated relaxation is not only caused by ROS neutralisation of NO, otherwise complete reversal would be expected. In other tissues, for example in aorta and mesenteric vessels, reversal of defective ACh-mediated relaxation by short-term exposure to antioxidants has been reported [45, 46]. In other experiments, short-term antioxidant treatment was, however, without effect on the diabetic deficit [11, 47]. This suggests that as well as a ROS neutralisation of NO, there are less readily reversible diabetic changes. Candidates include endothelial damage, a build-up of advanced glycation end products (AGEs) which can quench NO [13], the reaction between superoxide and NO produces peroxynitrite which can nitrosylate proteins and also decays to form nitrogen dioxide and the highly reactive hydroxyl radical [48], and changes in gene expression. The antioxidant LA has a number of beneficial actions to combat these changes in diabetes. Thus, LA scavenges ROS, prevents lipid peroxidation, improves tissue GSH content, attenuates the activation of nuclear factor κB when AGEs bind to their receptors, and protects against peroxynitrite-induced nitrosylation of tyrosine residues in proteins [25, 26, 49, 50]. There is no expectation that, once stimulated by diabetes, AGE formation, nitrosylation and changes in endothelial phenotypic expression mediated by nuclear factor κB, would be affected by LA treatment, therefore these factors could account for the poorly reversible component of the corpus cavernosum relaxation deficit.

The changes in corpus cavernosum autonomic control were specific; diabetes had a marked and early (4 weeks) effect on NANC fibres while sparing the noradrenergic innervation over the 8-week study period. It could be that a greater duration of diabetes would eventually lead to noradrenergic dysfunction; in the mesenteric vascular bed vasodilator nerve function is impaired earlier than the vasoconstrictor innervation [29]. The major neurotransmitter in corpus cavernosum NANC nerves is NO and diabetic effects on the nitrergic NANC system appear to be generalised, similar relaxation defects being reported for anococcygeus muscle, stomach and duodenum [51–53]. Treatment with LA completely prevented and partially reversed the corpus cavernosum nitrergic nerve deficit, in line with its effects on the endothelium NO system. This is the first report of successful treatment of a diabetic nitrergic deficit. It is likely that mechanisms causing nitrergic nerve dysfunction are similar to those suggested for the endothelium and that a superoxide, NO, peroxynitrite mechanism could be involved. Thus, NANC relaxation could possibly be abolished in vitro in the presence of a free radical generating system provided superoxide dismutase is inhibited [54].

Vasoactive intestinal polypeptide (VIP) is co-localised with NOS in neurones innervating corpus cavernosum smooth muscle [55] and it could potentially

have a role in NANC neurotransmission. Decreased sensitivity to exogenous VIP has been noted in diabetic rats [56], however, the relative importance of VIP is questionable. Intracavernosal injection does not produce a full erection in rats [57]. The effects of VIP are mediated by the cAMP system whereas in rat tissue and human cavernosum strips, NANC responses are blocked by NOS or guanylate cyclase inhibitors [58].

The diabetic deficits in corpus cavernosum ACh and NANC stimulated relaxations were not reproduced by acute exposure of nondiabetic tissue to high glucose concentrations. This has not previously been reported. In some other vascular tissues, effects to short-term glucose exposure are similar to those of diabetes. Thus, in rabbit and guinea pig aorta, high glucose effects are linked to ROS-stimulated endothelial release of a prostanoid vasoconstrictor which opposes ACh-induced relaxation [59, 60]. A similar effect was noted for rat mesenteric resistance vessels, combined with depression of endothelium derived hyperpolarising factor mediated responses [61]. Furthermore, cerebral and intestinal resistance vessels suffused with high glucose in vivo showed impaired responses to ACh, linked to prostanoid mechanisms, ROS and stimulation of protein kinase C [62, 63]. Like corpus cavernosum, responses of rat aorta were, however, unaltered by high glucose exposure [11], presumably because any modifications of prostanoid metabolism were minor and ACh relaxation is not mediated by endothelium derived hyperpolarising factor in these tissues.

In conclusion, diabetes has marked deleterious effects on corpus cavernosum relaxation in rats, similar to those found in human tissue. Thus, nitrergic NANC innervation and the endothelial NO system, which together can normally produced about 90% relaxation, have a joint diabetic deficit of about 40%. Treatment with the antioxidant, LA, completely prevented corpus cavernosum dysfunction and substantially (50–65%) corrected existing deficits. These data provide a rationale for the potential use of antioxidants in the treatment of diabetic impotence, as well as providing more general vascular benefits, which should be assessed in clinical trials.

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