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The effects of diabetes on nitric oxide-mediated responses in rat corpus cavernosum

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Abstract

Nitric oxide (NO)-mediated responses were investigated in corpora cavernosa isolated from 8-week diabetic rats. Relaxations to field stimulation were abolished by N^G-nitro-L-arginine (NOARG, 100 μM). Responses to stimulation and sodium nitroprusside were reduced in tissues from diabetic rats compared to control rats, when data were expressed as g tension, but not when expressed as g/g tissue. The endothelium-dependent vasodilator, acetylcholine, failed to relax tissues. Stimulation-induced contractions were smaller in the diabetic group compared to the control group when data were expressed as g tension, but not g/g tissue. Contractions were enhanced by NOARG, and inhibited by acetylcholine (300 μM), by a similar degree in both groups. NOARG reduced the inhibitory effect of acetylcholine in tissues from control, but not diabetic rats. The results suggest diabetes caused a general impairment in responsiveness of rat corpus cavernosum, which may be a consequence of tissue weight change. A role for endothelium-dependent NO could not be identified; however, NO-mediated modulation of noradrenergic transmission by acetylcholine, may be defective in diabetes. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Acetylcholine; Corpus cavernosum, rat; Nitrergic neurotransmission; Nitric oxide (NO); Nitric oxide (NO) synthase; Diabetes mellitus

1. Introduction

The development of penile tumescence leading to the erectile response requires enhanced blood inflow followed by the engorgement and filling of the sinusoidal spaces of the corpora cavernosa (Andersson and Wagner, 1995). Relaxation of the corporal smooth muscle is essential for this process to occur, and substantial evidence exists to implicate neuronal- and endothelial-derived nitric oxide (NO) in mediating this relaxant response (Sjöstrand and Klinge, 1995). Functional studies performed using isolated strips of rabbit and human corporal smooth muscle have identified relaxations induced by nonadrenergic, noncholinergic (NANC) neurotransmitters and muscarinic agonists, which were blocked by NO synthase inhibition, and which are dependent on both oxygen and cGMP (Ignarro et al., 1990; Holmquist et al., 1991a; Kim et al., 1991; Bush et al., 1992; Rajfer et al., 1992). A NO synthase-dependent erectile response was also identified in vivo following electrical stimulation of rabbit and rat cavernosal nerve (Holmquist et al., 1991b; Burnett et al., 1992), and dog pelvic nerve (Trigo-Rocha et al., 1993). Furthermore, NADPH-diaphorase staining and NO synthase immunoreactivity have been identified in nerves within the corporal smooth muscle from rat (Burnett et al., 1992; Keast, 1992; Dail et al., 1995), monkey (Okamura et al., 1998) and man (Burnett et al., 1993). The significant role for the NO pathway in penile physiology has therefore provided a focus for the identification of defects which may be involved in the pathophysiology of male sexual dysfunction.

Cardiovascular deterioration and neuropathy of autonomic nerves are established complications associated with the disease state diabetes mellitus (Hosking et al., 1978). Such alterations may underlie the high prevalence of impotence in diabetes, as up to 50% of diabetic men will develop this condition (Close and Ryder, 1995). Since neuronal- and endothelium-derived NO play an important role in erectile function, it is suggested that defects in the NO pathway may contribute to the erectile dysfunction caused by diabetes. Diabetes-induced impairments in NO-mediated, or nitrergic, neurotransmission have previously been identified in other smooth muscle preparations such as the rat anococcygeus, duodenum, gastric fundus and

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gastric body (Way and Reid, 1994; Jenkinson and Reid, 1995; Martinez-Cuesta et al., 1995; Takahashi et al., 1997). In addition, it is generally well established that the release of endothelium-derived NO is reduced by diabetes in vascular preparations (Tomlinson et al., 1992). Functional studies using isolated corpora cavernosa from diabetic men and alloxan-diabetic rabbits have revealed an impairment in both the neurogenic and endothelium-dependent mechanisms mediating smooth muscle relaxation (Saenz de Tejada et al., 1989; Azadzoi and Saenz de Tejada, 1992; Knispel et al., 1992). NO synthase immunoreactivity was also found to be reduced in penile tissue obtained from diabetic men with neurogenic impotence (Ehmke et al., 1995).

Other investigations using both chemically-induced and genetically diabetic rats have demonstrated diabetes adversely affects sexual/mating behavior (Stegar et al., 1989; Murray et al., 1992; Hassan et al., 1993; Elabbady et al., 1995; McVary et al., 1997), reflex erectile function (Murray et al., 1992; Vernet et al., 1995; McVary et al., 1997; Rehman et al., 1997) and the in vivo development of corporal pressure induce by electrical stimulation (Italiano et al., 1993; Rehman et al., 1997). However, normal sexual behavior and penile reflex has also been reported in male rats with long-term diabetes (Sachs et al., 1982). Specific studies to determine the effects of diabetes on the NO-biosynthetic/relaxation pathway in rat penile tissue have produced varying findings. Biochemical measurements have reported NO synthase activity to be both increased (Elabbady et al., 1995) and decreased (Vernet et al., 1995) in diabetic penile tissue. Stimulated cGMP synthesis is reportedly enhanced in diabetic penile tissue (Miller et al., 1994), as a consequence of reduced hydrolysis of cGMP by phosphodiesterase (Miller et al., 1996). Autoradiographic localisation revealed an increase in binding of NO synthase to the endothelium of the cavernosal space in diabetic tissue (Sullivan et al., 1996), whereas Western blot analyses indicated a reduction of penile neuronal NO synthase content in the diabetic group (Vernet et al., 1995). Together the majority of findings in the rat model of diabetes demonstrate that whole-animal sexual behavior, and in situ penile functioning is impaired by the diabetic state. Some biochemical and molecular analyses have identified defects in NO synthesis/availability which could lead to reduced NO generation and impaired tissue functioning, whereas other studies have demonstrated changes in the NO pathway which could lead to improved NO generation. It has been postulated that these latter findings may be indicative of an adaptive response of the NO pathway to counteract the diabetes-induced defects (Miller et al., 1994, 1996; Elabbady et al., 1995; Sullivan et al., 1996).

To date, there is little or no information in the literature examining the effects of diabetes on the functioning of rat isolated corporal smooth muscle in vitro. The aim of the present study was to use isolated corpus cavernosum from 8-week streptozotocin-diabetic rats, to investigate specifically the effect of diabetes on the functioning of neuronal- and endothelial-dependent responses mediated by NO.

2. Materials and methods

2.1. Induction of diabetes

Male Sprague–Dawley rats (200–250 g) were randomized into two groups. Diabetes was induced in one group by a single tail vein injection of streptozotocin (65 mg/kg). For 48 h after streptozotocin injection, rats received drinking water containing 2% sucrose, to alleviate the severity of the hypoglycaemic phase following treatment. The other control group received a single injection of citrate saline vehicle (20 mM, pH 4.5). Both treatment groups were maintained for an 8-week duration, during which all rats received food and normal drinking water ad libitum. Successful induction of diabetes was assessed in streptozotocin-treated rats by the reduction in weight gain, the appearance of glucosuria (Tes-Tape urine sugar analysis paper, Eli Lilly, Australia), polyuria and polydipsia. At the end of the 8-week treatment period, rats were killed by carbon dioxide asphyxiation, followed by decapitation. Samples of blood were collected into heparinised vials for the measurement of blood glucose levels (Ames Glucometer 3, Bayer Diagnostics, Australia).

2.2. Preparation of rat corpus cavernosum

Rat corpora cavernosa were isolated following the technique described by Italiano et al. (1994). Following decapitation, the whole penis was rapidly removed from the rat and placed in physiological salt solution (PSS; see below for composition), which was chilled and oxygenated. The organ was trimmed of connective tissue and the enlarged tip of the tissue (glans penis) removed. Fine dissection was performed with the aid of a dissection microscope, in order to clear the ventral and dorsal portions of the tissue. This involved the clearing of the corpus spongiosum with urethra, blood vessels and nerves, to expose the tough fibrous envelop (tunica albuginea) surrounding the cavernous bodies. Finally, the ventral and dorsal sections of the tunica albuginea were carefully removed, leaving an intact strip of tunica along each side of the penile shaft. Segments of the remaining tunica albuginea were dissected from each side of the strip as outlined by Italiano et al. (1994). A single preparation (approximately 4×13 mm^2) was obtained from each rat; this consisted of two segments of corpus cavernosum divided centrally by a septum, and partially surrounded by tunica albuginea. Partial removal of the tunica was necessary as reduced functioning of the isolated tissue was observed if left intact (Italiano et al., 1994; Way and Reid, unpublished observations).

Isolated tissues were mounted vertically in an 8-ml organ bath containing PSS of the following composition (mM; pH 7.4): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.03, $MgSO_4$ 0.45, D-(+)-glucose 5.5, disodium edetate 0.067, and ascorbic acid 0.14. The PSS was gassed constantly with 95% O_2/5% CO_2 and maintained at 37°C. Isometric recordings were obtained using a Grass FTO3C force–displacement transducer connected to a MacLab data acquisition system. Tissue tension was applied incrementally using 0.2 g increments every 15 min. The tension was increased until the magnitude of the response obtained to noradrenaline (1 μM) differed by no more than 10% of its previous size (Saenz de Tejada and Ignarro, 1996). Final tensions ranged from 0.8 to 1.0 g. All drugs were added directly into the organ bath. Field stimulation was applied through two platinum wire electrodes one on each side of the tissue, using square wave pulses of 1-ms duration and supramaximal voltage (15 V/cm) delivered from a Grass S88 stimulator. At the end of all experiments, tissues were carefully blotted and then weighed.

2.3. Experimental protocols

Relaxant responses were obtained in the presence of atropine (3 μM) and guanethidine (70 μM) in order to block stimulation-induced contractile responses, and to isolate the NANC neurotransmitter component. Tissues were initially contracted to cumulative additions of noradrenaline (0.01–100 μM); the concentration required to produce 75% of the maximum noradrenaline response was subsequently used to precontract tissues. Relaxant responses were obtained to field stimulation (1–32 Hz, 8-s train) delivered in random order at 1-min intervals, and to cumulative additions of sodium nitroprusside (0.01–300 μM). Relaxant responses to field stimulation were obtained before and after exposure to tetrodotoxin (3 μM, 30 min), or to the NO synthase inhibitor N^G-nitro-L-arginine (NOARG; 100 μM, 20 min); appropriate time-control studies were carried out in the absence of these agents. In separate experiments performed in the absence of guanethidine and atropine, acetylcholine was applied to precontracted tissues, as 1 mM additions every 2 min for a 20-min period (refer to Dail et al., 1987). Responses to bradykinin (0.03–3 μM) and carbachol (applied as for acetylcholine) were also assessed.

In the absence of atropine and guanethidine, contractile responses were obtained to field stimulation (1–32 Hz, 8-s train) delivered in random order at 1-min intervals, and to cumulative additions of noradrenaline (0.01–100 μM). Contractile responses were obtained before and after exposure to tetrodotoxin (3 μM, 30 min), or to NOARG (100 μM, 20 min); appropriate time-control studies were carried out in the absence of these drugs. In another series of experiments, contractions to field stimulation were obtained before and immediately after exposure to acetylcholine (300 μM), using tissues which were preincubated for at least 20 min in either the absence or presence of NOARG (100 μM). The ability of atropine (3 μM) to reverse the effects of acetylcholine was assessed in tissues in the absence of NOARG.

2.4. Drugs and drug solutions

Atropine sulphate, carbachol, guanethidine sulphate, NOARG, (−)-noradrenaline bitartrate, sodium nitroprusside, streptozotocin and tetrodotoxin were all purchased from Sigma (St. Louis, MO, USA). *O*-Acetylcholine perchlorate was obtained from BDH Chemicals (Poole, UK), and bradykinin from Auspep, Australia.

2.5. Statistical analyses

Results are shown as means ± S.E.M. and *n* indicates the number of animals tested. Differences between means were determined using Student's *t*-test, or by One- or Two-way analysis of variance (ANOVA), followed by the Student–Newman–Keuls test. The statistical software package Sigmastat (Jandel Scientific Software, CA, USA) was used to perform analyses. Probability levels less than 0.05 were taken to indicate statistical significance.

3. Results

3.1. Assessment of the effects of diabetes induction

Diabetes was confirmed in streptozotocin-treated rats by the appearance of glucosuria, polyuria and polydipsia. At the end of the 8-week treatment period, final blood glucose levels obtained from streptozotocin-treated rats were significantly greater ($P < 0.05$, unpaired Student's *t*-test) than

Table 1
Blood glucose levels, body weights and tissue weights of vehicle-treated rats and streptozotocin-treated rats. Values are means ± S.E.M. for the number of animals indicated in parentheses

Treatment group	Blood glucose[a] (mM)	Initial body weight (g)	Final body weight[a] (g)	Tissue weight[a] (mg)
Vehicle	5.4 ± 0.1 (27)	219 ± 3 (27)	482 ± 11 (27)	71.6 ± 2.1 (27)
Streptozotocin	24.0 ± 0.6[b] (30)	211 ± 3 (30)	261 ± 7[b] (30)	42.4 ± 1.6[b] (30)

[a]Measurements were obtained from rats 8 weeks after initial treatment with vehicle or streptozotocin.
[b]Significant difference from corresponding vehicle value ($P < 0.05$, unpaired Student's *t*-test).

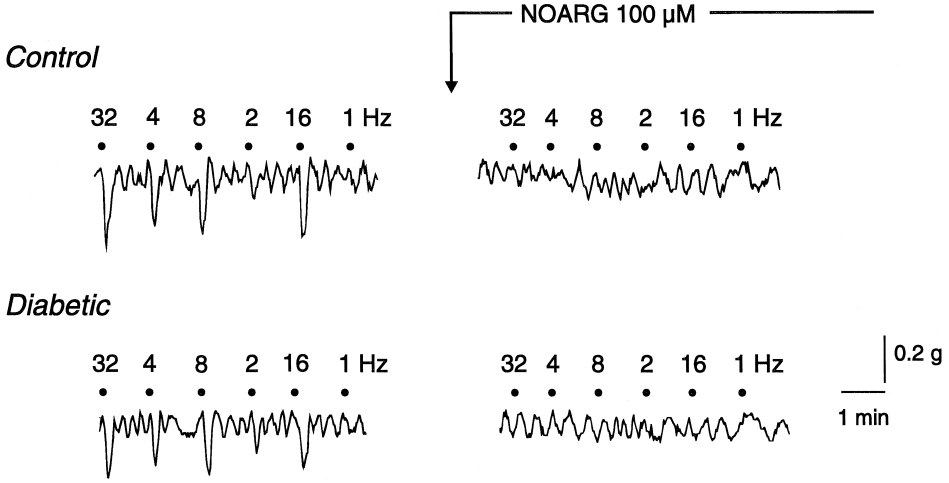

Fig. 1. Representative traces showing relaxant responses to field stimulation (●, 1–32 Hz, 8-s train) in corpus cavernosum from an 8-week control and diabetic rat, before and after exposure to NOARG (20 min). Tissues were incubated with guanethidine (70 μM) and atropine (3 μM), and precontracted with noradrenaline (1 μM for examples shown).

levels obtained from vehicle-treated rats (Table 1). Final body weights of streptozotocin-treated rats were significantly reduced ($P < 0.05$, unpaired Student's *t*-test) compared to body weights of vehicle-treated animals; initial body weights of the two treatment groups did not differ significantly ($P > 0.05$, unpaired Student's *t*-test; Table 1). Tissue weights of the corpora cavernosa obtained at the end of the experiment were significantly smaller ($P < 0.05$, unpaired Student's *t*-test) for streptozotocin-treated rats than for vehicle-treated rats (Table 1).

3.2. Relaxant responses

Precontraction levels to noradrenaline (75% of maximum response, see Section 2.3) in corpora cavernosa obtained from control rats (0.3–3 μM, 0.61 ± 0.06 g, $n = 8$) were significantly greater ($P < 0.05$, unpaired Student's *t*-test) than those obtained from the diabetic group (1–3 μM, 0.36 ± 0.04 g, $n = 7$). In the presence of guanethidine and atropine, field stimulation (1–32 Hz, 8-s train) of the corpus cavernosum produced frequency-dependent relaxant responses which were abolished by tetrodotoxin (3 μM, 30 min; data not shown), and by NOARG (100 μM, 20 min; Fig. 1). Relaxant responses obtained to field stimulation in tissues from diabetic rats were significantly reduced ($P < 0.05$, Two-way ANOVA), compared to responses obtained from control rats, when data were expressed as absolute reductions in g tension (Fig. 2A). Representative traces (Fig. 1) show a relatively high level of spontaneous/random activity between relaxant responses; this activity corresponds to a high noise/signal ratio because the recording system is approaching the limitations of sensitivity.

In order to normalise the data to account for differences in tissue weight between the two treatment groups, relaxations were also expressed as g relaxation/g tissue weight. After expression of relaxant responses to field stimulation as g/g tissue weight, relaxations did not significantly differ between the control and diabetic groups ($P > 0.05$, Two-way ANOVA; Fig. 2B). Similarly, relaxant responses obtained to sodium nitroprusside in tissues from diabetic

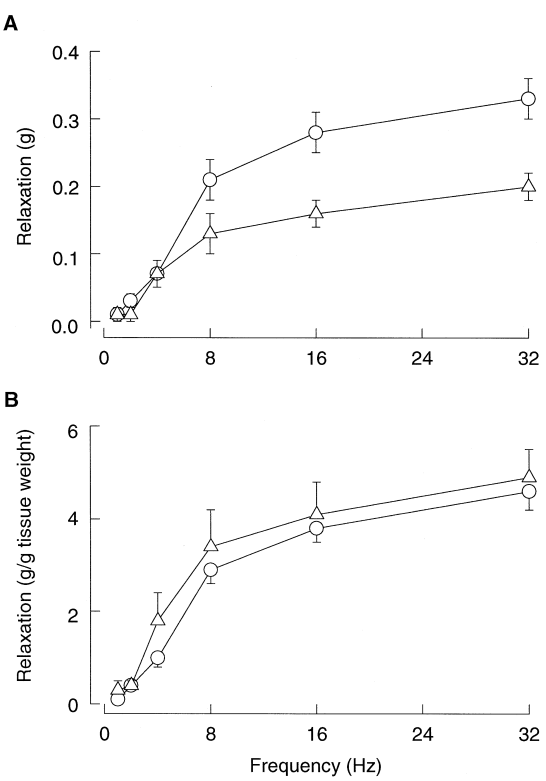

Fig. 2. Relaxant responses to field stimulation (1–32 Hz, 8-s train) in corpora cavernosa from 8-week control (○) and diabetic (△) rats. Data are means ± S.E.M. from seven to eight experiments, expressed as (A) absolute reductions in g tension, or (B) g relaxation/g tissue weight. Tissues were incubated with guanethidine (70 μM) and atropine (3 μM), and precontracted with noradrenaline.

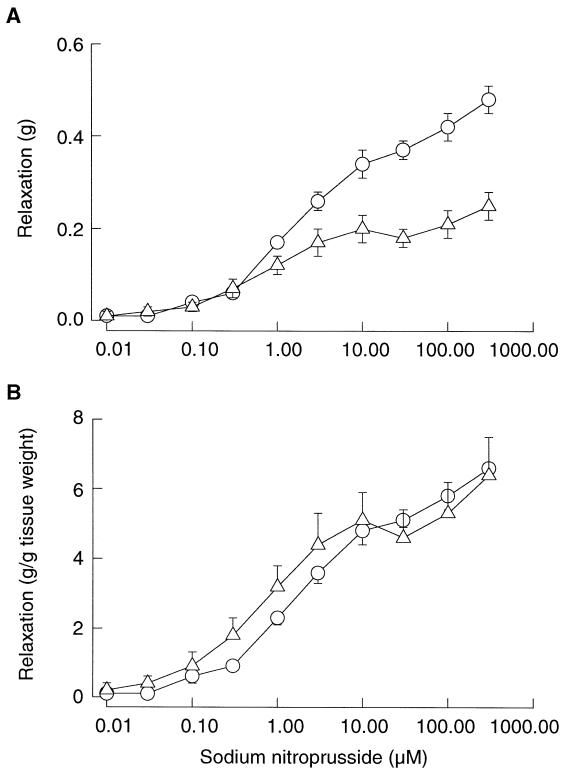

Fig. 3. Relaxant responses to sodium nitroprusside in corpora cavernosa from 8-week control (○) and diabetic (△) rats. Data are means ± S.E.M. from seven to eight experiments expressed as (A) absolute reductions in g tension, or (B) g relaxation/g tissue weight. Tissues were incubated with guanethidine (70 μM) and atropine (3 μM), and precontracted with noradrenaline.

rats were significantly reduced ($P < 0.05$, Two-way ANOVA) compared to responses from control rats, when expressed in g tension (Fig. 3A), but not when expressed as g/g tissue weight ($P > 0.05$, Two-way ANOVA; Fig. 3B).

In the absence of guanethidine and atropine, endothelium-dependent relaxations to acetylcholine were assessed in precontracted tissues. Single doses of acetylcholine at concentrations up to 1 mM failed to alter tissue tension. Furthermore, when acetylcholine (1 mM) was applied as repeated doses at 2-min intervals, following the method of Dail et al. (1987), little or no change in tissue tension was observed (data not shown). Similarly, carbachol (additions of 1 mM), and bradykinin (0.03–3 μM) did not change tissue tension in precontracted corpus cavernosa (data not shown).

3.3. Contractile responses

In the absence of guanethidine and atropine, field stimulation (1–32 Hz, 8-s train) of corpora cavernosa produced frequency-dependent contractile responses (Fig. 4), which were abolished by tetrodotoxin (3 μM, 30 min; data not shown). Contractile responses to 1, 2, 4, 8, 16 and 32 Hz stimulation in muscles from diabetic rats (0.01 ± 0.01, 0.02 ± 0.01, 0.04 ± 0.01, 0.10 ± 0.02, 0.23 ± 0.03, 0.35 ± 0.07 g, respectively, $n = 4$), were significantly smaller ($P < 0.05$, Two-way ANOVA) than the corresponding responses from control rats (0.01 ± 0.01, 0.03 ± 0.01, 0.08 ± 0.02, 0.16 ± 0.04, 0.44 ± 0.07, 0.63 ± 0.11 g, respectively, $n = 5$). When the data were normalised to account for differences in tissue weights between the treatment groups, the frequency–response curves were not significantly different ($P > 0.05$, Two-way ANOVA; Fig. 5).

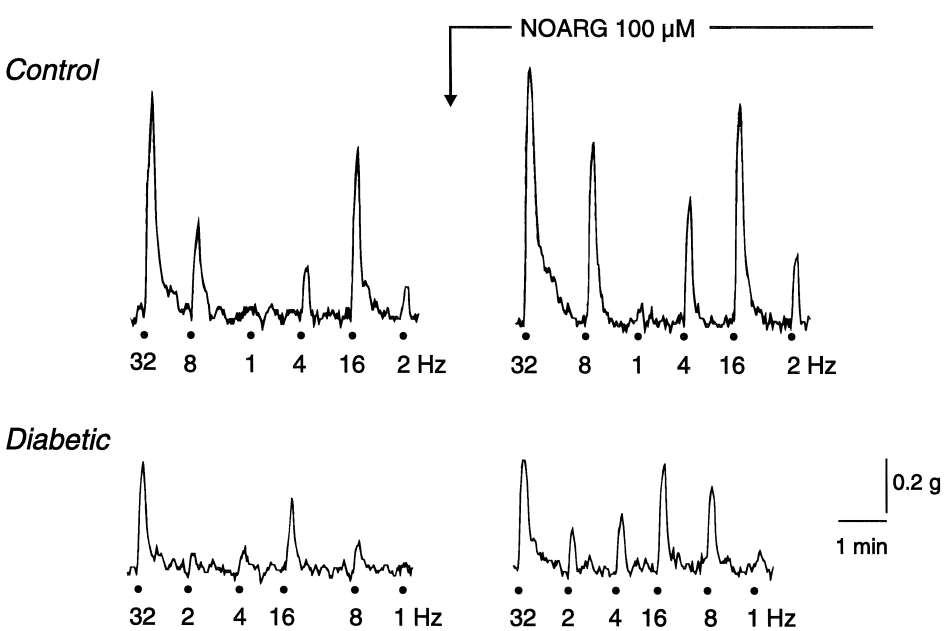

Fig. 4. Representative traces showing contractile responses to field stimulation (●, 1–32 Hz, 8-s train) in corpus cavernosum from an 8-week control and diabetic rat, before and after exposure to NOARG (20 min).

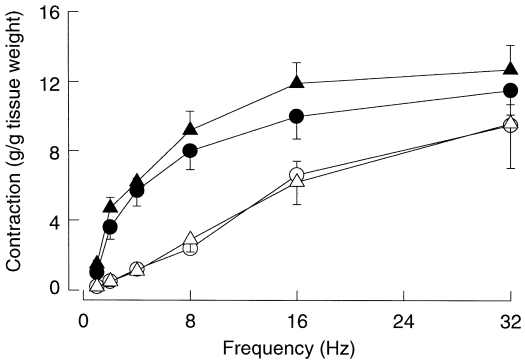

Fig. 5. Contractile responses to field stimulation (1–32 Hz, 8-s train) in corpora cavernosa from 8-week control (○) and diabetic (△) rats in the absence of NOARG, and from control (●) and diabetic (▲) rats after exposure to NOARG (100 μM, 20 min). Values are means ± S.E.M. from four to five experiments, expressed as g contraction/g tissue weight.

Exposure of tissues to NOARG (100 μM, 20 min) significantly enhanced ($P < 0.05$, Two-way ANOVA) contractile responses at frequencies of 1–16 Hz compared with corresponding time-control responses (Figs. 4 and 5). Furthermore, the degree of enhancement produced by NOARG was similar between the treatment groups, as contractile responses (expressed as g contraction/g tissue weight) obtained after exposure to NOARG did not differ significantly ($P > 0.05$, Two-way ANOVA) between the two treatment groups (Fig. 5).

Contractile responses obtained to cumulative additions of noradrenaline (0.01–100 μM) were significantly greater ($P < 0.05$, Two-way ANOVA) in tissues from control rats compared to those from the diabetic group, when expressed as absolute increases in g tension (Fig. 6A). However, when responses were expressed as g/g tissue weight, contractions did not differ significantly between the treatment groups ($P > 0.05$, Two-way ANOVA; Fig. 6B). Exposure to NOARG (100 μM) did not significantly ($P > 0.05$, Two-way ANOVA) alter contractions obtained to noradrenaline, in tissues obtained from either control or diabetic rats (Fig. 6).

The effects of acetylcholine were also examined on contractions to field stimulation. Acetylcholine (300 μM) significantly inhibited ($P < 0.05$, Two-way ANOVA) contractile responses in corpora cavernosa from both control (Fig. 7A) and diabetic rats (Fig. 7B), in an atropine (3 μM)-reversible manner (data not shown). To compare the inhibitory effect produced by acetylcholine between the treatment groups, responses obtained after exposure to acetylcholine were expressed as percent of the initial response obtained before acetylcholine addition. No signifi-

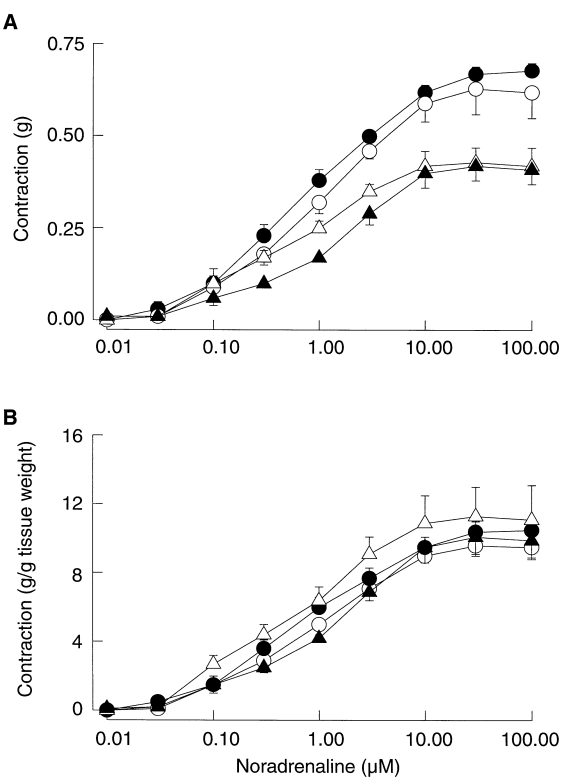

Fig. 6. Contractile responses to noradrenaline in corpora cavernosa from 8-week control (○) and diabetic (△) rats in the absence of NOARG, and from control (●) and diabetic (▲) rats after exposure to NOARG (100 μM, 20 min). Values are means ± S.E.M. from four to five experiments, expressed as (A) absolute increases in g tension, and (B) g contraction/g tissue weight.

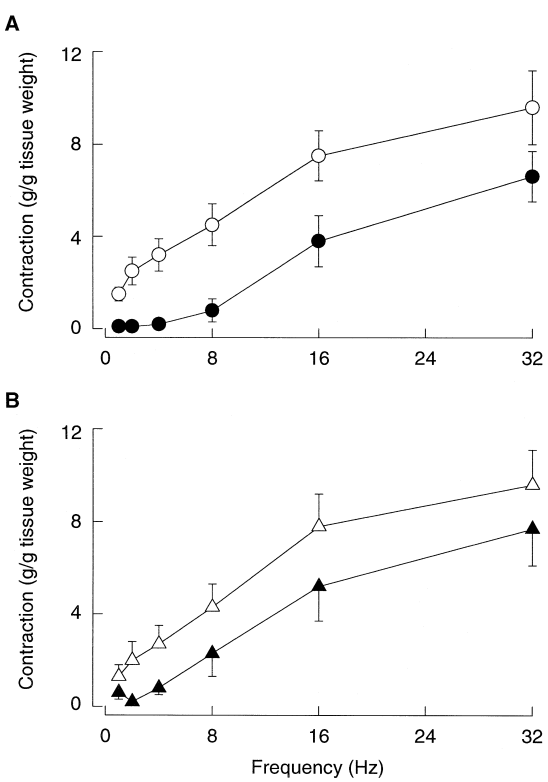

Fig. 7. Contractile responses to field stimulation (1–32 Hz, 8-s train) in corpora cavernosa from 8-week (A) control rats before (○) and after (●) exposure to acetylcholine (300 μM), and (B) diabetic rats before (△) and after (▲) acetylcholine exposure. Values are means ± S.E.M. from five to eight experiments, expressed as g/g tissue weight.

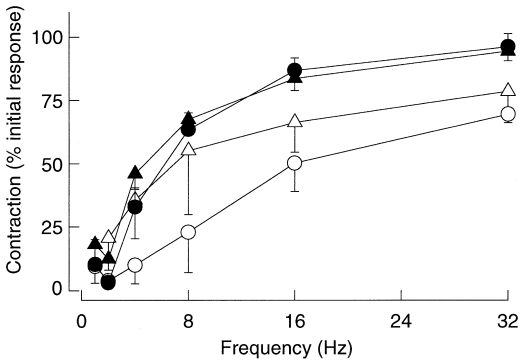

Fig. 8. Contractile responses to field stimulation (1–32 Hz, 8-s train) in corpora cavernosa from 8-week control (○) and diabetic (△) rats showing the inhibitory effect of acetylcholine (300 μM) alone, and in control (●) and diabetic (▲) rats in the presence of NOARG (100 μM). Values are means ± S.E.M. from five to eight experiments, expressed as a percentage of the initial response obtained before the addition of acetylcholine.

cant difference was apparent ($P > 0.05$, Two-way ANOVA) between the inhibition produced by acetylcholine in tissues from control rats, compared to that obtained from diabetic rats (Fig. 8). The inhibitory effect of acetylcholine was also assessed in the presence of NOARG (100 μM). NOARG significantly reduced ($P < 0.05$, Two-way ANOVA) the inhibitory effect of acetylcholine on contractions to field stimulation in tissues from control rats, whereas NOARG had no significant effect ($P > 0.05$, Two-way ANOVA) on the ability of acetylcholine to inhibit contractile responses in tissues from diabetic rats (Fig. 8).

4. Discussion

In the present study, corpora cavernosa with a small amount of intact tunica albuginea were isolated from 8-week control and diabetic rats following the method of Italiano et al. (1994). Electrical stimulation of corporal smooth muscle under NANC conditions produced frequency-dependent relaxations which were confirmed to be neurogenic in origin, and entirely NO-mediated, or nitrergic in nature, since they were abolished by tetrodotoxin and NOARG, respectively. Stimulation-induced relaxations were significantly reduced in tissues from 8-week diabetic rats; a reduction in response size to the NO donor, sodium nitroprusside, was also apparent. These findings suggest that diabetes attenuates nitrergic neurotransmission in rat corpus cavernosum via an impairment in smooth muscle reactivity. However, the streptozotocin-induced diabetic rat model produces a hyperglycaemic state which is also accompanied by a reduction in both body and tissue weight. Although reduced corporal weight observed in the diabetic group may be associated with reduced weight gain, it is also reported that reduced serum levels of the androgen testosterone, which occurs in this model of diabetes, can lead to impaired growth of the male sex organs (Öztürk et al., 1996). Interestingly, the erectile response in the rat is dependent on androgens, which are reported to modulate the expression and activity of penile NO synthase (Penson et al., 1996; Reilly et al., 1997; Schirar et al., 1997). As the impairment in functioning of corporal smooth muscle from diabetic rats could be a consequence of reduced tissue size, data were re-analysed to account for tissue differences between the treatment groups. It was noted that both tissue weight and levels of precontraction for corpora cavernosa from diabetic rats were reduced to approximately 59% of control values. Therefore, similar results were obtained whether data were expressed as g/g tissue weight or as a percent of precontraction; the former method of data expression was chosen as this method could also be used for expression of contractile data. Following this method of data expression, the difference in tissue function between the control and diabetic groups was no longer apparent, suggesting that the diabetes-induced reduction in tissue weight contributes to impaired functioning. It is not clear from this study why reduced tissue weight should impair tissue functioning, or if the reduction in tissue weight per se rather than a diabetes-induced defect is responsible. This issue could be clarified in additional studies utilizing weight-matched control rats, which may also display equivalent decreases in tissue weight.

Our findings in rat corpus cavernosum are not consistent with the previously reported effects of experimental diabetes examined in vitro using rabbit corporal smooth muscle. An impairment in the neurogenic relaxation of corpus cavernosum, without alterations in smooth muscle reactivity was demonstrated using 6-week alloxan-diabetic rabbits (Azadzoi and Saenz de Tejada, 1992). In contrast to the present study, numerous strips of corpus cavernosum were dissected from each rabbit without the tunica albuginea, the preparations were similar in tissue weight, and the level of phenylephrine precontraction did not differ between the treatment groups (Azadzoi and Saenz de Tejada, 1992). Similar dissection methods would be difficult to follow in the rat due to the small tissue size, and removal of the entire tunica may also increase the possibility of damage to the smooth muscle and endothelium which could reduce tissue viability. Although the effect of diabetes on tissue weight also differs between species, the method used in this study to normalise data should sufficiently take such variations into account. Studies performed using 8-week streptozotocin-diabetic rats in vivo, have observed an impairment in the development of intracorporal pressure induced by electrical stimulation of the cavernous nerve (Rehman et al., 1997). In contrast to our studies using isolated corpus cavernosum in vitro, the specific effects of diabetes on the nitrergic nerve component are not determined in vivo, as the final response observed results from the stimulation of all erectile nerve components within the tissue, which may or may not be

altered by diabetes. Furthermore, in vivo experiments cannot take into account any differences in tissue size, which may influence function. To allow for a direct comparison of our in vitro findings with whole tissue function, additional in vivo studies using 8-week streptozotocin-diabetic rats to examine penile reflex, stimulation-induced intracorporal pressure, or sexual behavior, would be necessary. An in vivo study would also provide confirmation that the 8-week duration of diabetes used in this study is sufficient for the development of defects in rat erectile function, as it has been previously reported that diabetes-induced changes may be time-dependent (MacLeod and McNeill, 1985).

Another aim of the present study was to investigate the effect of diabetes on endothelium-derived NO function in rat corpus cavernosum. Functional investigations performed on isolated corporal tissue from rabbit and man have reported acetylcholine-induced relaxations to be impaired by diabetes (Saenz de Tejada et al., 1989; Azadzoi and Saenz de Tejada, 1992). In the present study, application of acetylcholine to rat corporal smooth muscle failed to produce tissue relaxation, even at high concentrations greater than 1 mM; similar results were obtained with carbachol and bradykinin. The findings in the present study are partly in accord with those reported by Dail et al. (1987), who found that single doses of acetylcholine failed to produce relaxation, although repeated acetylcholine addition at high concentrations (final concentration 10 mM) eventually induced significant relaxation of the rat corporal tissue. A lack of responsiveness to acetylcholine in corporal tissue could suggest that damage to the endothelium may have occurred during tissue preparation, and this could be confirmed histochemically; however, endothelial removal from corporal tissue is reported to be technically difficult, due to the complex arrangement/anatomy of cavernosal tissue (Ignarro et al., 1990). Alternatively, the results of the present study could indicate that release of NO from the endothelium does not play an important role in rat corpus cavernosum function, suggesting that the streptozotocin-treated rat may not be a suitable model to investigate the effects of diabetes on the endothelial NO pathway in the corpus cavernosum. In support of the latter proposal, Miller et al. (1994) failed to detect acetylcholine-stimulated cGMP synthesis in both control- and diabetic-rat corpus cavernosum, and suggested that the acetylcholine/NO pathway may not be present in the penis of the rat. Histochemical studies of rat penile tissue have described NADPH-diaphorase and NO synthase-immunohistochemical staining of the endothelium lining the cavernosal spaces either to be present in some groups of endothelial cells (Keast, 1992), to be faint (Burnett et al., 1992), or to be absent (Schirar et al., 1994; Dail et al., 1995).

Release of noradrenaline from noradrenergic nerves of the penis is believed to contribute to the maintenance of flaccidity, and to the production of detumescence (Andersson and Wagner, 1995). In the absence of noradrenergic and cholinergic blockade, field stimulation of rat corpus cavernosum produced frequency-dependent contractions which appeared to be reduced in tissues obtained from 8-week diabetic rats; responses to noradrenaline were also reduced, suggesting a defect in smooth muscle reactivity. However, normalisation of the data to account for tissue weight, revealed no difference in reactivity to either field stimulation or noradrenaline between the two groups. This suggests that reduced tissue weight which accompanies diabetes, impairs tissue functioning, rather than specifically affects noradrenergic neurotransmission.

Exposure to the NO synthase inhibitor NOARG, significantly enhanced contractile responses to field stimulation, but not to exogenous noradrenaline, indicating that concurrent release of NO from nitrergic nerves modulates noradrenergically mediated responses. The amount of enhancement produced by NOARG did not differ between the treatment groups, suggesting that the ability of NO released from nitrergic nerves to modulate the noradrenergic response was not altered by diabetes. This provides additional evidence to suggest that nitrergic nerve functioning is not altered by diabetes in rat corpus cavernosum.

Noradrenergically mediated contractions in rat isolated corpus cavernosum are reported to be modulated by acetylcholine. In the present study, acetylcholine partially inhibited stimulation-induced contractions and this could be reversed by atropine, similar to the findings reported by Dail et al. (1987). However, no difference in the inhibitory action of acetylcholine was observed between tissues from control and diabetic rats. The mechanisms by which acetylcholine acts to reduce noradrenergically mediated responses in rat corporal smooth muscle are unknown. It has been suggested that the inhibitory effects of acetylcholine in penile tissue could include (i) muscarinic suppression of excitatory noradrenergic neurotransmission, (ii) muscarinic stimulation of endothelium-derived NO, and (iii) nicotinic-induced release of a NANC relaxant factor (Sjöstrand and Klinge, 1995). The third mechanism can be eliminated in the present study, since the inhibitory effect of acetylcholine was blocked by atropine. To differentiate between the first two mechanisms, the contribution of NO in mediating acetylcholine-induced inhibition in the present study was examined by preincubating tissues with NOARG. The inhibitory effect of acetylcholine on stimulation-induced contractions was significantly reduced in control tissues exposed to NOARG, indicating a large component of its effect is mediated by NO and a smaller component is NO synthase-independent. It is not clear whether the NO involved in acetylcholine modulation, is derived from the endothelium or from nitrergic nerves. Our findings provided no evidence to suggest that acetylcholine is able to stimulate NO from the endothelium to produce relaxation of rat corporal smooth muscle and, therefore, it is unlikely that endothelium-derived NO is involved. However, further studies in which the endothelium is removed, or in which a specific inhibitor of either the endothelial or

neuronal NO synthase isoform is used, would be required to help identify the contribution of neuronal- and endothelium-derived NO to the modulation of contractile responses by acetylcholine. In contrast to the control group the inhibitory effect of acetylcholine in tissues from diabetic rats was not altered by NOARG exposure, suggesting that little or no NO contributes to its inhibitory action in diabetic tissues. Alternatively, NO may have no effect on noradrenaline release from noradrenergic nerves in diabetic tissues. This latter possibility may be clarified by assessment of the effects of NO on noradrenaline release in corpora cavernosa from control and diabetic rats. Therefore, the effect of acetylcholine to inhibit stimulation-induced contractions in diabetic tissues may involve prejunctional muscarinic suppression of noradrenergic neurotransmission only.

5. Conclusion

This study has examined the effects of diabetes on tissue function in an in vitro preparation of rat corpus cavernosum. In contrast to previous reports using corpus cavernosum from other species, a role for endothelium-dependent NO function could not be identified in the rat. On the other hand, relaxations to nerve stimulation obtained under NANC conditions were entirely mediated by NO. Diabetes reduced relaxant responses to both nitrergic nerve stimulation and sodium nitroprusside, but the reductions were negated when responses were corrected for changes in tissue weight, suggesting that the differences in nitrergic neurotransmission may simply be a consequence of tissue weight change. Evidence was obtained to suggest that NO released from nitrergic nerves is involved in the modulation of noradrenergic transmission. NO is also involved in acetylcholine-mediated modulation of noradrenergic transmission; this involvement was absent in diabetic tissue, suggesting that this modulatory role of NO may be defective in diabetes.

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