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The effect of sildenafil on corpus cavernosal smooth muscle relaxation and cyclic GMP formation in the diabetic rabbit

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Abstract

Sildenafil, a type V phosphodiesterase inhibitor, enhances smooth muscle relaxation in normal human and rabbit corpus cavernosum. We investigated the in vitro effects of sildenafil on non-adrenergic, non-cholinergic and nitric oxide (NO)-mediated cavernosal smooth muscle relaxation in diabetic rabbits, since alterations in this pathway are recognised in diabetic erectile dysfunction. Diabetes mellitus was induced in male New Zealand White rabbits with alloxan. Cavernosal strips from age-matched control, 3- and 6-month diabetic animals were mounted in organ baths. Relaxation responses to electrical field stimulation (1–20 Hz) or sodium nitroprusside (10^{-8}–10^{-4} M) were assessed in the absence and presence of sildenafil (10^{-8} and 10^{-7} M). The effect of sildenafil on cGMP formation by the corpus cavernosum was also assessed following stimulation with sodium nitroprusside, A23187 and acetylcholine. Sodium nitroprusside-stimulated relaxations were significantly ($P < 0.03$) impaired in the corpus cavernosum from both diabetic groups, ($IC_{50} = 4.6 \times 10^{-6}$ M following 3 months of diabetes mellitus and 4.0×10^{-6} M following 6 months of diabetes mellitus; compared to 7.5×10^{-7} M for pooled age-matched controls). Sildenafil (10^{-7} M) significantly enhanced sodium nitroprusside-stimulated relaxation in control ($P < 0.05$) and diabetic groups ($P < 0.03$). Electrical field stimulation-mediated relaxations of the corpus cavernosum were significantly impaired after 6-month diabetes mellitus and enhanced by sildenafil (10^{-8} M). cGMP formation by the diabetic corpus cavernosum was impaired significantly, but restored towards normal by sildenafil. We suggest that the impairment of NO-mediated relaxation of the corpus cavernosum reflect, at least in part, a defect in guanylyl cyclase activity. These findings support the use of sildenafil as an effective, orally administered, treatment for diabetic erectile dysfunction.

Keywords: Sildenafil; Corpus cavernosum; Diabetes mellitus; Electrical field stimulation; cGMP

1. Introduction

It is now widely accepted that the risk factors that underlie the development of vascular disease are identical to those that predispose to vasculogenic erectile dysfunction (Virag et al., 1984; Krane et al., 1989; Azadzoi and De Tejada, 1991, 1992; Shabsigh et al., 1991; Azadzoi and Goldstein, 1992; Lerner et al., 1993). A major risk factor for both atherogenesis and erectile dysfunction is diabetes mellitus (De Tejada et al., 1989; Blanco et al., 1990). The prevalence of erectile dysfunction in diabetic men is as high as 50% (Krane et al., 1989; Blanco et al., 1990; Lerner et al., 1993).

Non-adrenergic, non-cholinergic (NANC) neurotransmission plays an important role in mediating penile erection (Kim et al., 1991; De Tejada, 1992; Trigo-Rocha et al., 1993; Finberg et al., 1993). Nitric oxide (NO) released by the endothelium of the arteries that supply the penis as well as the corpus cavernosum and NANC neurotransmission mediate corpus cavernosal smooth muscle relaxation through the formation of guanosine 3′5′cyclic monophosphate (cGMP) (Bredt and Snyder, 1994). This NANC drive is adversely affected by diabetes mellitus (De Tejada et al., 1989) with subsequent impairment of corpus caver-

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nosal relaxation. This may be due, at least in part, to the development of diabetic neuropathy, which has been shown to contribute to neurogenic erectile dysfunction (Krane et al., 1989; Blanco et al., 1990; Lerner et al., 1993).

Previously, the pharmacological treatment of erectile dysfunction has been confined to intra-cavernosal (Virag, 1982; Von Heyden et al., 1993; Shenfeld et al., 1995) and transurethral (Padma-Nathan et al., 1997) injections of drugs such as papaverine and prostaglandin E_1.

Recent clinical trials, however, have demonstrated that the oral administration of sildenafil is an effective form of treatment for erectile dysfunction (Eardley et al., 1996; Boolell et al., 1996; Gingell et al., 1996), even in diabetic men (Rendell et al., 1999). Sildenafil is a type V cGMP phosphodiesterase inhibitor that enhances NO-induced cGMP formation/accumulation resulting in a significant relaxation of the corpus cavernosum (Ballard et al., 1996; Jeremy et al., 1997). It has also been suggested that the sildenafil-induced enhancement of cGMP accumulation is dependent upon a priori endogenous NANC and parasympathetic drive (Jeremy et al., 1997). Since neuropathy can be a complication of diabetes mellitus, it could also be argued that sildenafil may be less effective in diabetic patients if this drive is diminished. In order to test these possibilities, the effect of sildenafil on relaxation and cGMP formation by the corpus cavernosum of the diabetic rabbit and age-matched controls was studied, ex vivo. Since the angiopathic and neuropathic effects of diabetes mellitus are time dependent, we studied corpus cavernosal relaxation responses to sildenafil at both 3 and 6 months after the induction of diabetes mellitus.

2. Materials and methods

Acetylcholine, calcium ionophore A23187, guanethidine, indomethacin, atropine, phenylephrine, sodium nitroprusside and alloxan were purchased from Sigma (Poole, Dorset, UK): [^{125}I] cGMP kits were purchased from Amersham Radiochemicals (Amersham, UK). Krebs solution (KRB; pH 7.4) had the following composition (mM): 118 NaCl, 4.6 KCl, 25 $NaHCO_3$, 1.2 $MgSO_4 \cdot 7H_2O$, 1.2 KH_2PO_4, 2.5 $CaCl_2$ and 11.0 glucose. Pfizer (Central Research, Sandwich Kent, UK) provided sildenafil citrate.

2.1. Induction of DM

Age-matched 3 kg male New Zealand white rabbits were injected intravenously with alloxan (via the ear vein; 65 mg/kg). The diabetic rabbits were fed ad libitum with SDS standard rabbit plain (SDS, Whitham, UK) and allowed free access to water.

2.2. Collection and analysis of samples

Blood was sampled at monthly intervals from the ear artery. The blood was placed in serum gel bottles to determine serum electrolytes, creatinine, urea, cholesterol, triglyceride and glucose concentrations using standard methodology for the Hitachi 717 Automatic Autoanalyzer (Boehringer Mannheim, Lewes, Sussex, UK).

Urine was also monitored over the duration of diabetes for glucose, ketone bodies and proteins with Multistix (Ames Division, Miles Laboratories, Stoke Poges, Buckinghamshire, UK) (Sullivan et al., 1998).

2.3. Organ bath studies

At 3 and 6 months, age-matched controls and diabetic rabbits ($n = 6$ in all groups) were killed by cervical dislocation and penises rapidly excised and placed in cold oxygenated Krebs solution at 4 °C. Tissue preparations were investigated on the same day of acquisition. Epidermal tissue was removed and the tunica albuginea opened and the corpus cavernosum dissected out, cut into strips of approximately 1×3 mm. The size and weights of the tissues from both control and diabetic rabbits were similar. The strips were mounted vertically in 1.5-ml capacity organ baths, equipped with two parallel platinum electrodes for electrical field stimulation. The tissues were bathed with Krebs solution at pH 7.4, maintained at 37 °C by a thermoregulated circuit and bubbled with a mixture of $95\%O_2$–$5\%CO_2$. This solution also contained guanethidine (5×10^{-6} M), atropine (10^{-5} M) and indomethacin (10^{-6} M) to inhibit the adrenergic, cholinergic and cyclooxygenase pathways, respectively, leaving the NANC pathway intact. An initial tension of 2 g was applied to the suspended tissue strips. The tension was recorded on a Grass Polygraph (model 7D; Astro-med Grass, Slough UK). All strips were equilibrated for at least 1 h. At the end of the equilibration period, the strips were challenged with KCl (124 mM). Two reproducible contractions varying in magnitude by less than 10% were consistently obtained. Tissues were then pre-contracted with phenylephrine (10^{-4} M). Transmural electrical field stimulation of nerves were performed with a Grass S88 (Astro-med Grass, Slough UK) stimulator delivering single square waves (duration 0.8 ms) over a range of frequencies that gave an incremental increase in the relaxation response (1–20 Hz) in 5-s trains at 2-min intervals. A series of relaxations in response to electrical field stimulation in the absence of sildenafil were recorded, this was repeated in the presence of sildenafil (10^{-8} M) after a 15-min incubation period.

In other studies, tissues were pre-contracted with phenylephrine (10^{-4} M) and cumulative response curves were constructed for sodium nitroprusside (10^{-8}–10^{-4} M). The tissues were washed several times over a 1-h period and re-contracted with phenylephrine (10^{-4} M). Sildenafil (10^{-7} M) was then added to the organ bath and after 15 min cumulative response curves were again constructed for sodium nitroprusside.

Table 1
Weight changes (kg) in the age-matched controls, 3 and 6 months diabetic rabbits

Animal	Body weight (kg)	
	Starting	Final
3 months age-matched control	3.33 ± 0.03	4.15 ± 0.17[a]
3 months diabetic	3.35 ± 0.08	3.58 ± 0.24
6 months age-matched control	3.40 ± 0.02	4.70 ± 0.04[b]
6 months diabetic	3.51 ± 0.05	3.28 ± 0.10[c,d]

Results are expressed as mean ± S.E.M. Number of animals in each group, $n = 6$.

[a] 3 months age-matched control starting weight vs. final weight (Student's paired *t*-test): $P < 0.002$.

[b] 6 months age-matched control starting weight vs. final weight (Student's paired *t*-test): $P < 0.0001$.

[c] 6 months age-matched control final weight vs. 6 months diabetic final weight (Student's unpaired *t*-test): $P < 0.0001$.

[d] 6 months diabetic starting weight vs. 6 months diabetic final weight (Student's unpaired *t*-test): $P < 0.04$.

2.4. Cyclic GMP formation

In parallel experiments corpus cavernosum from 3 to 6 months diabetic rabbits and their age-matched controls were incubated with different concentrations of sildenafil (10^{-8}–10^{-6} M). The formation of cGMP was then stimulated with calcium ionophore A23187 (10^{-5} M), acetylcholine (10^{-5} M) and sodium nitroprusside (10^{-6} M) (which breaks down in aqueous solution to generate NO). After 20 min incubation at 37 °C in a water bath, reactions were stopped with 1 M perchloric acid. The preparation was then ultrasonicated (3×20 s burst; MSE Soniprep, Fisher Scientific, Loughborough, UK). After centrifugation, aliquots of supernatant were taken and neutralised with 0.5 M K_3PO_4 and then acetylated with acetic anhydride: triethylamine (2:1, v/v). cGMP concentrations were then measured using a specific radioimmunoassay (Jeremy et al., 1997).

2.5. Statistical analysis

Comparisons of animal weight and serum biochemical indices were performed between the 3- and 6-month diabetic groups and the pooled age-matched controls using Student's paired or unpaired *t*-test.

Isolated corpus cavernosal strips responses to sodium nitroprusside in the absence or presence of sildenafil are expressed as % relaxation of phenylephrine-induced tone. All results are expressed as mean ± S.E.M. for at least 12 separate strips from 6 animals in each experimental group (age-matched control groups were pooled). Comparisons were made using Student's *t*-test for unpaired samples and by analysis of variance (ANOVA) with statistical significance accepted with $P < 0.05$.

For the cGMP measurements data are related to mg/mg tissue/min. Each data point is expressed as mean ± S.E.M. in triplicate with six animals in each group. Comparisons were made using Student's *t*-test for unpaired samples and statistical significance was accepted with $P < 0.05$.

3. Results

3.1. Animal weights

The starting weights of the age-matched controls, 3- and 6-month diabetic rabbits were all similar. At the end of 3-month diabetes mellitus, the final weights were not significantly different from their starting weights. However, in the 6 months diabetic group, the final weights were significantly decreased. Age-matched controls gained a significant amount of weight at 3 and 6 months when compared to their starting weight. There was no significant difference between the final weight of the age-matched control and the 3-month diabetic animals. In contrast, at 6 months, the age-matched controls were significantly heavier than the 6-month diabetic animals (Table 1).

3.2. Serum biochemical indices

Serum sodium, chloride, creatinine, urea and glucose concentrations (non-fasting) were significantly elevated in the 3-month and 6-month diabetic rabbits when compared to pooled age-matched controls. In contrast, cholesterol and triglyceride concentrations were not elevated in either diabetic group when compared to pooled age-matched controls (Table 2).

Table 2
Changes in serum sodium, chloride, urea, creatinine, glucose, cholesterol and triglyceride (TG) concentrations in pooled age-matched controls, 3 and 6 months diabetic rabbits

Biochemical variables	Pooled age matched control ($n = 12$)	3 months diabetic ($n = 6$)	6 months diabetic ($n = 6$)
Sodium (mmol/l)	141.6 ± 0.38	132.5 ± 1.30[a]	132.4 ± 1.02[a]
Chloride (mmol/l)	101.5 ± 0.97	88.47 ± 2.22[a]	91.75 ± 1.77[a]
Glucose (mmol/l)	8.30 ± 0.28	29.6 ± 1.96[a]	29.5 ± 1.34[a]
Urea (mmol/l)	6.34 ± 0.28	9.37 ± 0.58[a]	11.13 ± 0.56[a]
Creatinine (μmol/l)	92.5 ± 2.38	105.7 ± 4.07[b,c]	116.1 ± 2.12[a]
Cholesterol (mmol/l)	1.39 ± 0.31	0.79 ± 0.23	0.77 ± 0.09
Triglyceride (mmol/l)	1.96 ± 0.36	1.77 ± 0.47	1.33 ± 0.36

Pooled age-matched controls vs. 3 or 6 months diabetic.

[a] $P < 0.0001$ (Student's unpaired *t*-test). 3months diabetic vs. 6 months diabetic.

[b] $P < 0.009$.

[c] $P < 0.04$ (Student's *t*-test). Number of animals in parentheses.

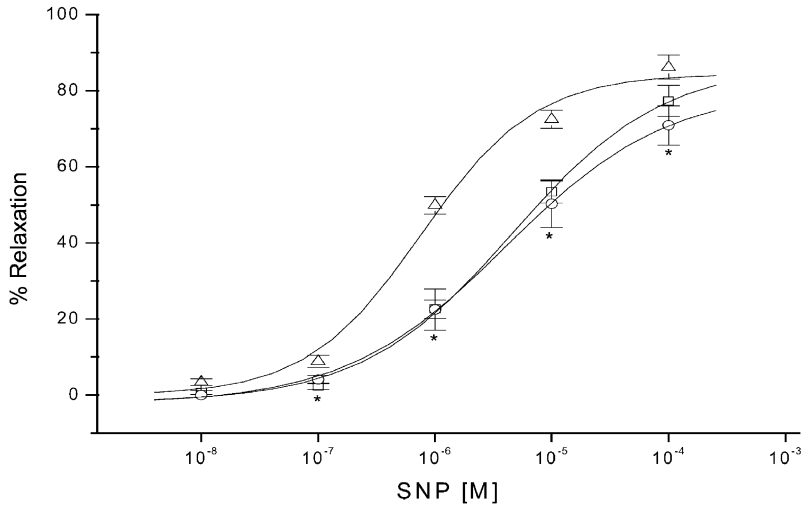

Fig. 1. Sodium nitroprusside (SNP)-mediated relaxation of corpus cavernosal strips taken from age-matched control (△), 3 months (□) and 6 months (○) diabetic rabbits. The strips were precontracted with phenylephrine (10^{-4} M) and cumulative response curves were constructed for SNP (10^{-8}–10^{-4} M). Results are expressed as % relaxation of the phenylephrine-induced tone. SNP-mediated relaxation of 3 and 6 months diabetic corpus cavernosal strips were significantly impaired when compared to the pooled age-matched control strips $P < 0.03$; ANOVA test. * Denotes a significant difference ($P < 0.05$) in SNP-mediated relaxation between age-matched controls and 3 or 6 months diabetic cavernosal strips at each data point using a Student's unpaired *t*-test. Number of animals in the pooled age-matched controls $n = 12$ and $n = 6$ in both diabetic groups.

Serum biochemical variables (except creatinine) were not significantly different between the 3-month and 6-month diabetic groups.

3.3. Urinary biochemical indices

Urinary ketone and protein concentrations were not significantly different between age-matched controls and both diabetic groups (results not shown).

3.4. Relaxation studies

Sodium nitroprusside-stimulated relaxation was impaired in the corpus cavernosum from 3 months diabetic rabbits ($IC_{50} = 4.6 \times 10^{-6}$ M) and 6 months diabetic rabbits ($IC_{50} = 4.0 \times 10^{-6}$ M) compared to pooled 3 and 6 months age-matched controls ($IC_{50} = 7.5 \times 10^{-7}$ M) (Fig. 1). The 3- and 6-month age-matched control data were pooled since we found no difference in the IC_{50} between

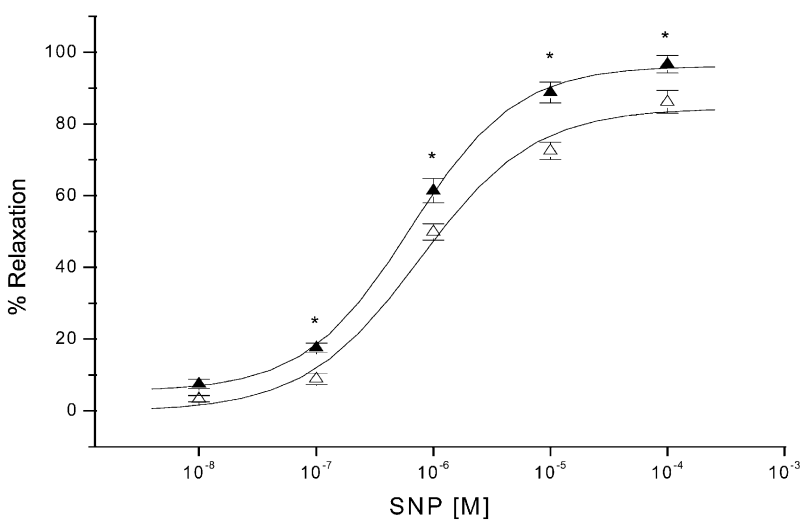

Fig. 2. The effect of sildenafil on sodium nitroprusside (SNP)-mediated relaxation of corpus cavernosal strips taken from pooled age-matched control rabbits. The strips were precontracted with phenylephrine (10^{-4} M) and cumulative response curves were constructed for SNP (10^{-8}–10^{-4} M). The tissues were washed several times over a 1-h period and re-contracted with phenylephrine (10^{-4} M). Sildenafil (10^{-7} M) was then added to the organ bath and after 15 min cumulative response curves were again constructed for SNP. Results are expressed as % relaxation of the phenylephrine-induced tone in the absence (△) and presence of sildenafil (▲). Sildenafil significantly enhanced the SNP-mediated corpus cavernosal relaxations $P < 0.05$; ANOVA test. * Denotes a significant difference ($P < 0.05$) at each data point using a Student's unpaired *t*-test. Number of animals $n = 12$.

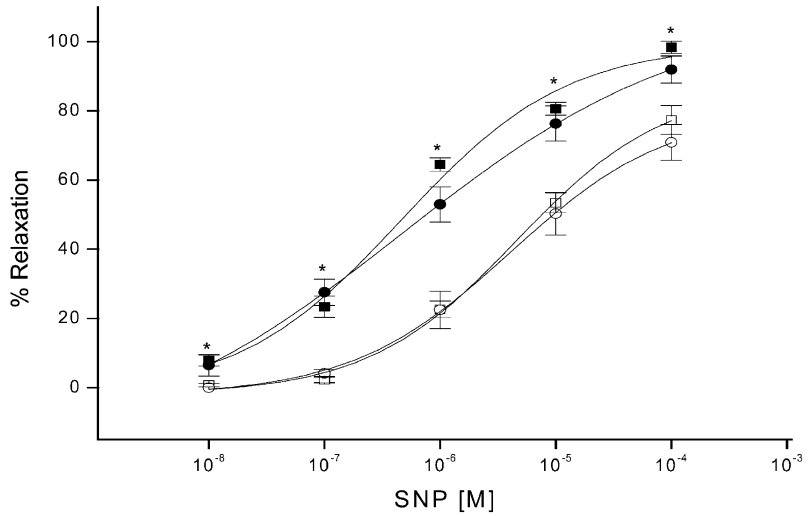

Fig. 3. The effect of sildenafil on sodium nitroprusside (SNP)-mediated relaxation of corpus cavernosal strips taken from 3 and 6 months diabetic rabbits. The strips were precontracted with phenylephrine (10^{-4} M) and cumulative response curves were constructed for SNP (10^{-8}–10^{-4} M). The tissues were washed several times over a 1-h period and re-contracted with phenylephrine (10^{-4} M). Sildenafil (10^{-7} M) was then added to the organ bath and after 15 min cumulative response curves were again constructed for SNP. Results are expressed as % relaxation of the phenylephrine-induced tone in the absence (□) and presence of sildenafil (■) for 3 months diabetic cavernosal strips and in the absence (○) and presence of sildenafil (●) for 6 months diabetic cavernosal strips. Sildenafil significantly enhanced the SNP-mediated corpus cavernosal relaxations for both 3 and 6 months diabetic rabbits $P < 0.03$; ANOVA test. * Denotes a significant difference ($P < 0.05$) in SNP-mediated relaxation between the absence and presence of sildenafil, at each data point, in both diabetic groups using a Student's unpaired *t*-test. Number of animals in both diabetic groups $n = 6$.

both groups. Sildenafil (10^{-7} M) significantly enhanced sodium nitroprusside-stimulated corpus cavernosal relaxations from pooled age-matched controls, as well as, 3 and 6 months diabetic rabbits (Figs. 2 and 3), whilst having no effect on phenylephrine-induced corpus cavernosal tone.

There were no significant differences in electrical field stimulation-induced corpus cavernosal relaxations between 3 months diabetic rabbits and the pooled age-matched control (results not shown). However, after 6 months diabetes, electrical field stimulation-induced relaxations

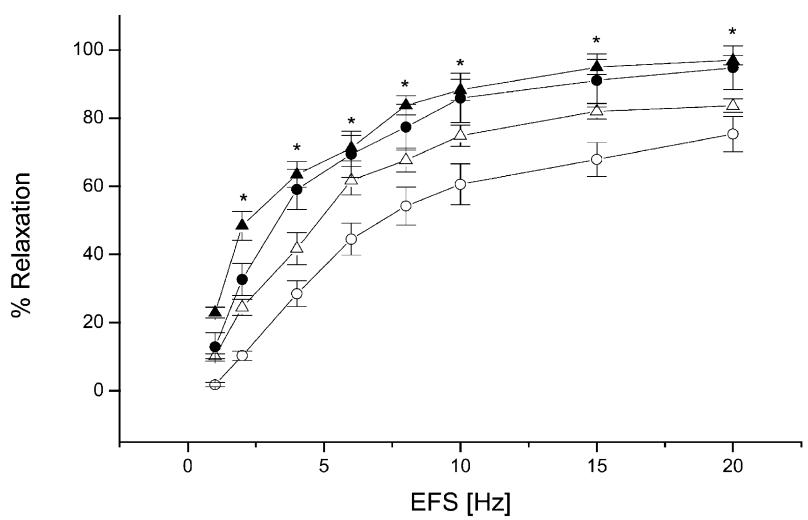

Fig. 4. The effect of sildenafil on NANC-mediated relaxation of corpus cavernosal strips taken from 6 months diabetic and age-matched control rabbits. The strips were mounted in organ baths equipped with platinum electrodes for electrical field stimulation (EFS). The strips were precontracted with phenylephrine (10^{-4} M) and stimulated over a range of frequencies (1–20 Hz). Sildenafil (10^{-8} M) was then added to the organ bath and after 15 min EFS were repeated. Results are expressed as % relaxation of the phenylephrine-induced tone in the absence (○) and presence of sildenafil (●) for 6 months diabetic cavernosal strips and in the absence (△) and presence of sildenafil (▲) for pooled age-matched control cavernosal strips. EFS-induced relaxations were significantly impaired when comparing 6 months diabetic to pooled age-matched control strips $P < 0.03$; ANOVA test. Sildenafil significantly enhanced the EFS-stimulated corpus cavernosal relaxations in 6 months diabetic and pooled age-matched control rabbits $P < 0.03$ and $P < 0.05$, respectively; ANOVA test. * Denotes a significant difference ($P < 0.05$) in EFS-stimulated relaxation between the absence and presence of sildenafil, at each data point, in 6 months diabetic and age-matched control rabbits using a Student's unpaired *t*-test. Number of animals in the pooled age-matched control and 6 months diabetic groups $n = 12$ and $n = 6$, respectively.

Table 3
Effect of sildenafil on cGMP formation by 3 and 6 months diabetic and control corpus cavernosal tissue stimulated with sodium nitroprusside
(A) Corpus cavernosal tissues were taken from 3 months diabetic and 3 months control rabbits and incubated with different concentrations of sildenafil (0–10^{-6} M). The formation of cGMP was then stimulated with sodium nitroprusside (10^{-6} M). cGMP was measured using a specific radioimmunoassay and expressed as cGMP produced/mg of tissue/min. (B) Corpus cavernosal tissue taken from 6 months diabetic and 6 months control rabbits under went a similar experimental procedure as the 3 month diabetic and control rabbits.

Sildenafil concentration (M)	fmoles cGMP/mg tissue/min	
	Controls	3 months diabetic
A		
0	2.7 ± 0.	0.6 ± 0.2[a]
10^{-8}	4.0 ± 1.0	1.7 ± 0.6[a]
10^{-7}	11.1 ± 2.	8.3 ± 1.9
10^{-6}	16.8 ± 1.8	14.5 ± 2.6
B		
0	3.0 ± 0.4	0.5 ± 0.3[a]
10^{-8}	4.4 ± 0.6	1.3 ± 0.4[a]
10^{-7}	11.0 ± 2.0	7.5 ± 1.8
10^{-6}	17.3 ± 2.6	14.8 ± 2.1

[a] Denotes a significant difference ($P < 0.001$) in cGMP formation at each data point between diabetic and control tissues using a Student's unpaired *t*-test. Number of animals in each group, $n = 6$.

were significantly impaired (Fig. 4). Sildenafil (10^{-8} M) significantly enhanced electrical field stimulated corpus cavernosal relaxations in 6 months diabetic rabbits, as well as pooled age-matched controls (Fig. 4)

3.5. cGMP formation

The formation of cGMP in response to sodium nitroprusside was significantly reduced in the corpus cavernosum from both 3 and 6 months diabetic rabbits compared to age-matched controls (Table 3). cGMP formation was also reduced when A23187 and acetylcholine were used as stimulators in both diabetic groups (results not shown). Sildenafil enhanced the formation of cGMP in response to all stimulators in control and both diabetic groups. At high sildenafil (10^{-7} and 10^{-6} M) concentrations, there were no significant differences between both diabetic groups and age-matched control tissues (see Table 3 for sodium nitroprusside results).

4. Discussion

Although the usefulness of sildenafil in treating diabetic erectile dysfunction has been highlighted (Rendell et al., 1999), the present study demonstrates, for the first time, that sildenafil at therapeutic concentrations enhances NO-dependent corpus cavernosal smooth muscle relaxation as well as cGMP formation in diabetic rabbits compared to age-matched controls.

Both sodium nitroprusside-stimulated corpus cavernosal relaxation and cGMP formation were impaired in the 3- and 6-month diabetic rabbits. Since sodium nitroprusside dissociates in solution to generate NO, which in turn activates guanylyl cyclase, these results suggest that diabetes mellitus impairs the activity of this enzyme causing a reduction in cGMP production. This finding is in contrast, however, to an earlier study that showed that cGMP formation was enhanced in the corpus cavernosum from diabetic rats (Miller et al., 1994). We ascribe this difference to species variation and the severe catabolic status of the diabetic rats (Thompson and Mikhailidis, 1992). The diabetic rat is not an ideal model to use in the present study, since unlike the diabetic rabbit and man, it has elevated high density lipoprotein levels and is not prone to atherosclerosis which is also an established risk factor for erectile dysfunction (Virag et al., 1984; Krane et al., 1989; Azadzoi and De Tejada, 1991).

Sildenafil enhanced sodium nitroprusside-stimulated relaxation of corpus cavernosal tissue from pooled age-matched control and diabetic rabbits. These findings are in agreement with a previous in vitro study showing that sildenafil enhanced NO-mediated corpus cavernosal relaxation of healthy rabbits (Ballard et al., 1996). To our knowledge, there is no previous data showing that sildenafil enhances relaxation of the corpus cavernosum following diabetes mellitus.

Sildenafil is a selective and potent phosphodiesterase type V inhibitor that inhibits the hydrolysis of cGMP, thereby elevating levels of this cyclic nucleotide (Jeremy et al., 1997). Our findings therefore demonstrate that type V phosphodiesterase activity is intact in diabetic corpus cavernosal tissue. As sildenafil normalised the reduced sodium nitroprusside-mediated relaxations of diabetic corpus cavernosum, we suggest that the actions of this drug can compensate for the diabetes-induced impairment of guanylyl cyclase activity. Thus, the diminished levels of cGMP in diabetic corpus cavernosal tissue may not only be due to impaired guanylyl cyclase activity but also enhanced phosphodiesterase activity.

The sildenafil-induced enhancement of corpus cavernosal relaxation was considerably more pronounced in the diabetic group compared to the age-matched control. This implies that the reduced cGMP formation by the diabetic corpus cavernosum causes an increase in sensitivity to cGMP.

Sodium nitroprusside-stimulated cGMP formation was impaired in the diabetic groups compared to age-matched controls. Similar results were obtained when either acetylcholine or A23187 was used to stimulate cGMP formation. Stimulation by acetylcholine and A23187 is dependent on NO release by the activation of endothelial nitric oxide synthase (eNOS) leading to endothelium-dependent NO-mediated smooth muscle relaxation. These data imply that

apart from impaired guanylyl cyclase activity, there could also be a defect in eNOS activity as previously suggested (Sullivan et al., 1998). Sildenafil overcame the diabetic impairment of A23187-, acetylcholine- and sodium nitroprusside-stimulated cGMP formation such that responses were not significantly different between diabetic and control tissues.

In a previous study, we showed that sodium nitroprusside-stimulated cGMP formation was not significantly different between age-matched control and diabetic rabbit corpus cavernosum (Sullivan et al., 1998). However, there are methodological differences between the present study and the earlier one. The earlier study was carried out in the presence of isobutyl methylxanthine, an inhibitor of phosphodiesterase activity and the data adjusted by the subtraction of corpus cavernosal basal cGMP production. In the present study, however, isobutyl methylxanthine was not included in the incubation buffer so that basal cGMP production could be determined. As cGMP production was reduced in diabetic corpus cavernosum in this study, it implies that the hydrolysis of cGMP by phosphodiesterase determines basal cGMP level. This concurs with a previous study using corpus cavernosum from diabetic rats, which showed that diminished hydrolysis of cGMP caused elevated cGMP levels (Miller et al., 1994). This conclusion would also be consistent with the effect of sildenafil on cGMP formation from diabetic rabbit corpus cavernosum: namely, inhibition of type V phosphodiesterase activity.

It is now accepted that NANC neurotransmission plays an important, if not a key role in mediating penile erection (Kim et al., 1991; De Tejada, 1992; Trigo-Rocha et al., 1993; Finberg et al., 1993). In turn, there is evidence that diabetes mellitus adversely affects the NANC drive in man (De Tejada et al., 1989). One of the principal neurotransmitters released by NANC neurones is NO, which causes the formation of cGMP and corpus cavernosal relaxation (Bredt and Snyder, 1994).

We found a significant impairment in electrical field stimulation-mediated corpus cavernosal relaxation after 6 months but not after 3 months of diabetes mellitus. Since electrical field stimulation elicits relaxation through activation of the NANC neurones that innervate the corpus cavernosum, these data suggests that diabetic neuropathy may have developed after 6 months but not 3 months of diabetes mellitus. There is evidence to support this supposition, since early signs of vascular neuropathy, characterised by a reduction in the neuronal content and release of noradrenalin by sympathetic nerves is evident in rabbits 6 weeks after the induction of diabetes mellitus (Cohen et al., 1990). It is therefore conceivable that with time, the biochemical changes could cause some degree of neuropathy. In turn, this could lead to the impairment of the NANC pathway, resulting in erectile dysfunction. Reduced relaxation to electrical field stimulation in corpus cavernosal tissue taken from diabetic patients with erectile dysfunction has also been reported (Pickard et al., 1994). Interestingly, this was associated with a lack of NO production and not an inherent inability of the corpus cavernosum to relax.

A reduction in NO could result from a defect in NO synthesis and thus NANC neurotransmission or quenching of NO through the production of superoxide radicals and advanced glycosylation end-products (Ceriello et al., 1991; Hoffman et al., 1995). As these factors are thought to play a role in the pathogenesis of diabetic erectile dysfunction. Sildenafil enhanced the relaxation of the corpus cavernosum in response to electrical field stimulation in age-matched controls 3 and 6 months diabetic rabbits.

The present findings suggest that despite the early development of diabetic neuropathy, sildenafil still remains effective in eliciting corpus cavernosal smooth muscle relaxation, although we cannot predict how effective it would be if severe neuropathy is present. In such cases co-administration of a NO donor may compensate for the impairment of NO bioavailability, not only as a result of impaired NANC neurotransmission but also as a consequence of impaired corpus cavernosal endothelial function and diabetes-induced advanced glycosylation end-products or superoxide formation.

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References

Azadzoi, K.M., De Tejada, I.S., 1991. Hypercholesterolemia impairs endothelium–dependent relaxation of rabbit corpus cavernosum smooth muscle. J. Urol. 146, 238–240.

Azadzoi, K.M., De Tejada, I.S., 1992. Diabetes mellitus impairs neurogenic and endothelium-dependent relaxation of rabbit corpus cavernosum smooth muscle. J. Urol. 148, 1587–1591.

Azadzoi, K.M., Goldstein, I., 1992. Erectile dysfunction due to atherosclerotic vascular disease: the development of an animal model. J. Urol. 147, 1675–1681.

Ballard, S.A., Turner, L.A., Naylor, A.M., 1996. Sildenafil (Viagra™), a potent selective inhibitor of type 5 phosphodiesterase enhances nitric oxide-dependent relaxation of rabbit corpus cavernosum. Br. J. Pharmacol. 118, 153P.

Blanco, R., De Tejada, I.S., Goldstein, I., Krane, R.J., Wotiz, H.H., Cohen, R.A., 1990. Dysfunctional penile cholinergic nerves in diabetic impotent men. J. Urol. 144, 278–280.

Boolell, M., Allen, M.J., Ballard, S.A., Gepi-Allee, S., Muirhead, G.J., Naylor, A.M., Osterloh, I.H., Gingell, C., 1996. Sildenafil: an orally active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction. Int. J. Impot. Res. 8, 47–52.

Bredt, D.S., Snyder, S.H., 1994. Nitric oxide: a physiologic messenger molecule. Annu. Rev. Biochem. 63, 175–195.

Ceriello, A., Giugliano, D., Quatraro, A., Dello Russo, P., Lefebvre, P.V., 1991. Metabolic control may influence the increased superoxide generation in diabetic serum. Diabetes Med. 8, 540–542.

Cohen, R.A., Tesfamariam, B., Weisbrod, RM., Zitnay, K.M., 1990. Adrenergic denervation in rabbits with diabetes mellitus. Am. J. Physiol. 259, H55–H66.

De Tejada, I.S., 1992. Mechanisms for the regulation of penile smooth muscle. In: Lue, T.F. (Ed.), World Book of Impotence. Smith/Gordon, London, p. 39.

De Tejada, I.S., Goldstein, I., Azadzoi, K., Krane, R.J., Cohen, R.A., 1989. Impaired neurogenic and endothelium-mediated relaxation of penile smooth muscle from diabetic men with impotence. N. Engl. J. Med. 320, 1025–1030.

Eardley, I., Morgan, R.J., Dinsmore, W.W., Pearson, J., Wulff, M.B., Boolell, M., 1996. UK-92, 480, a new oral therapy for erectile dysfunction, a double blind placebo controlled trial with treatment taken as required. J. Urol. 155 (5 (Suppl)), 495A.

Finberg, J.P.M., Levy, S., Vardi, Y., 1993. Inhibition of nerve stimulation-induced vasodilation in corpora cavernosa of the pithed rat by blockade of nitric oxide synthase. Br. J. Pharmacol. 108, 1038–1042.

Gingell, C.J.C., Jardin, A., Olsson, A.M., Dinsmore, W.W., Osterloh, I.H., Kirkpatrick, J., Cuddingan, M., 1996. UK-92,480 a new oral therapy for erectile dysfunction, a double blind placebo controlled, once daily dose–response study. J. Urol. 155 (5 (Suppl)), 495A.

Hoffman, D., Seftel, A.D., Hampel, N., Resnick, M.I., 1995. Advanced glycation end-products quench cavernosal nitric oxide. J. Urol. 153, 441A.

Jeremy, J.Y., Ballard, S., Naylor, A., Miller, M.A.W., Angelini, G.D., 1997. Effect of sildenafil (Viagra™), a specific inhibitor of cGMP phosphodiesterase on cAMP and cGMP formation by the rabbit corpus cavernosa, in vitro. Br. J. Urol. 79, 958–963.

Kim, N., Azadzoi, K.M., Goldstein, I., De Tejada, I.S., 1991. A nitric oxide-like factor mediates nonadrenergic–noncholinergic neurogenic relaxation of penile corpus cavernosum smooth muscle. J. Clin. Invest. 88, 112–118.

Krane, R.J., Goldstein, I., De Tajeda, I.S., 1989. Impotence. N. Engl. J. Med. 321, 1648–1659.

Lerner, S.E., Melman, A., Christ, G.J., 1993. A review of erectile dysfunction: new insights and more questions. J. Urol. 149, 1246–1255.

Miller, M.A.W., Morgan, R.J., Thompson, C.S., Mikhailidis, D.P., Jeremy, J.Y., 1994. Adenylate and guanylate cyclase activity in the penis and aorta of the diabetic rat: an invitro study. Br. J. Urol. 74, 106–111.

Padma-Nathan, H., Hellstrom, J.G., Kaiser, F.E. et al., 1997. Treatment of men with erectile dysfunction with transurethral alprostadil. N. Engl. J. Med. 336, 1–7.

Pickard, R.S., King, P., Zar, M.A., Powell, P.H., 1994. Corpus cavernosal relaxation in impotent men. Br. J. Urol. 74, 485–491.

Rendell, M.S., Rajfer, J., Wicker, P.A., Smith, M.D., 1999. Sildenafil for treatment of erectile dysfunction in men with diabetes. A randomized control trial. JAMA 281, 421–426.

Shabsigh, R., Fishman, I.J., Schum, C., Dunn, J.K., 1991. Cigarette smoking and other vascular risk factors in vasculogenic impotence. Urology 38, 227–231.

Shenfeld, O., Hanani, J., Shalhav, A., Vardi, Y., Goldwasser, B., 1995. Papaverine-phentolamine and prostaglandin E_1 versus papaverine-phentolamine alone for intracorporeal injection therapy: a clinical double-blind study. J. Urol. 154, 1017–1019.

Sullivan, M.E., Thompson, C.S., Mikhailidis, D.P., Morgan, R.J., Angelini, G.D., Jeremy, J.Y., 1998. Differential alterations of prostacyclin, cyclic AMP and cyclic GMP formation in the coprus cavernsum of the daibetic rabbit. Br. J. Urol. 82, 578–584.

Thompson, C.S., Mikhailidis, D.P., 1992. Haematological profile and plasma and urinary biochemistry in experimental models of diabetes mellitus-predictive power for human studies. J. Pharm. Med. 2, 241–257.

Trigo-Rocha, F., Hsu, G.L., Donatucci, C.F., Lue, T.F., 1993. The role of cyclic adenosine monophosphate, cyclic guanosine monophosphate, endothelium and nonadrenergic, noncholinergic, neurotransmission in canine penile erection. J. Urol. 149, 872–877.

Virag, R., 1982. Intracavernosal injection of papaverine for erectile failure. Lancet 2, 938.

Virag, R., Bouilly, P., Frydman, D., 1984. Is impotence an arterial disorder? A study of arterial risk factors in 440 impotent men. Lancet 181–184.

Von Heyden, B., Donatucci, C., Kaula, N., Lue, T.F., 1993. Intracavernous pharmacotheraphy for impotence: selection of appropriate agent and dose. J. Urol. 149, 1288–1290.