

The Effect of The Specific Phosphodiesterase Type V Inhibitor, “Sildenafil Citrate”, on Alloxan-Induced Diabetic Rabbit Corpus Cavernosal Smooth Muscle

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Abstract

This study was carried out to investigate the effect of sildenafil (S) together with the influence of sodium nitroprusside (SNP) and acetylcholine (Ach) on isolated strips of rabbit corpus cavernosum. The in vitro effects of sildenafil on non-adrenergic, non-cholinergic and nitric oxide (NO)-mediated cavernosal smooth muscle relaxation in diabetic rabbits, were also investigated. Diabetes mellitus was induced in adult male New Zealand white rabbits with alloxan (65 mg/kg, i.v). Cavernosal strips from age-matched control and 5 months diabetic animals were mounted in organ baths containing Krebs-bicarbonate solution (pH 7.4, bubbled continuously with a gas mixture of 95% O₂ plus 5% CO₂, and maintained at 37°C. Isolated strips of control rabbit corpus cavernosum were stimulated isotonicly with phenylephrine (10⁻⁵M) and relaxations were induced using increasing concentrations of Ach, SNP and S alone and in combination. Also, relaxation responses of control and diabetic strips precontracted with phenylephrine (10⁻⁵M) to electrical field stimulation (EFS, 4-20 Hz) or SNP (10⁻⁹ – 10⁻³M) were assessed in the absence and presence of a submaximal dose of sildenafil (10⁻⁶M).

It was found that Ach, SNP and S relaxed the control rabbit corpus cavernosal strips in a dose-dependent manner. The relaxant effect of the test agents were ranked (from least inhibitory to most inhibitory) as follows: S, Ach plus S, SNP, SNP plus S, Ach. Moreover, it was observed that SNP-stimulated relaxations were significantly impaired in the corpus cavernosum from diabetic group compared to age-matched controls. Sildenafil (10⁻⁶M) significantly enhanced SNP-stimulated relaxation in control and diabetic groups. Electrical field stimulation-mediated relaxations of the corpus cavernosum were significantly impaired after 5 months diabetes mellitus and enhanced by sildenafil (10⁻⁶M).

In conclusion, sildenafil enhances the relaxing effect of both SNP and Ach on the phenylephrine-induced contraction of rabbit cavernosal tissue. It could also be suggested that the impairment of NO-mediated relaxation of the diabetic corpus cavernosum reflect, at least in part, a defect in guanyl cyclase activity. These findings support the use of sildenafil as an effective, orally administered, treatment for diabetic erectile dysfunction.

Introduction and Aim of the Work

Many studies have highlighted the pivotal role of nitric oxide (NO) and its stimulation of cyclic guanosine monophosphate (cGMP) in the mediation of the penile corpus cavernosal smooth muscle relaxation and erection (Kim *et al.*, 1991; De Tejada, 1992; Trigo-Rocha *et al.*, 1993; Finberg *et al.*, 1993; Burnet, 1995).

NO is synthesized in the nerve terminals of the parasympathetic non-adrenergic, noncholinergic neurons in the penis corpus cavernosum and also by the endothelial cells lining the blood vessels (arteries that supply the penis) and the lacunar space of the corpora cavernosa (Burnet *et al.*, 1992; Trigo-Rocha *et al.*, 1993; Bredt and Snyder, 1994). It activates guanylate cyclase, resulting in an increased conversion of guanosine triphosphate to cGMP. cGMP is an important intracellular messenger for transducing extracellular signals in corpus cavernosal smooth muscle cells (Burnet, 1995; Holmquist *et al.*, 1991). By binding to cGMP-dependent protein kinases and to cGMP-dependent ion channels, it reduces intracellular calcium levels (Meyer *et al.*, 1993). Reduced intracellular calcium levels result in trabecular smooth muscle relaxation, enabling corporal volume expansion (Moreland *et al.*, 1998; Carter *et al.*, 1998). The level of cGMP is regulated by a balance between the rate of synthesis and the rate of hydrolytic breakdown to cGMP by cyclic nucleotide phosphodiesterase (PDE) isoenzymes (Beavo, 1992). Therefore agents that inhibit cGMP hydrolysis may increase the cGMP signal and could be expected to enhance the relaxation of the smooth muscle in the corpus cavernosum and thereby facilitate penile erectile responses (Ballard *et al.*, 1998).

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; Azadzoï and DeTejada, 1991, 1992; Shabsigh *et al.*, 1991; Azadzoï 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 high (Krane *et al.*, 1989; Blanco *et al.*, 1990; Lerner *et al.*, 1993).

Nonadrenergic noncholinergic (NANC) drive is adversely affected by diabetes mellitus (De Tejada *et al.*, 1989) with subsequent impairment of corpus cavernosal relaxation. This may be due to, 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 intracavernosal (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 prostaglandine E₁.

Sildenafil is a selective phosphodiesterase type 5 inhibitor, which is widely used for the oral treatment of erectile dysfunction, regardless of etiology. It enhances NO-mediated cGMP – formation / accumulation resulting in a significant relaxation of the corpus cavernosum (Ballard *et al.*, 1996; Jeremy *et al.*, 1997). It effectively blocks phosphodiesterase type 5-mediated hydrolysis of cGMP. Data obtained from rabbit corpus cavernosal

tissues have demonstrated that sildenafil increases endogenous corpus cavernosal smooth muscle cGMP and induces relaxation of the muscles (Jeremy et al., 1997; Chuang *et al.*, 1988). It has also been suggested that the sildenafil-induced enhancement of cGMP accumulation is dependent on a priori endogenous NANC and parasympathetic drive (Jeremy *et al.*, 1997).

Therefore, the objectives of this study was to (1) examine the effects of sildenafil in the physiologically important NO-cGMP signal transduction pathway for relaxation of corpus cavernosal smooth muscle and to compare it with endothelial-dependent relaxation of acetylcholine and endothelial-independent relaxation of sodium nitroprusside. (2) test the possible effect of sildenafil on relaxation of the corpus cavernosum of the diabetic rabbits and age-matched controls.

Material & Methods

Induction of diabetes mellitus:

Adult male white rabbits (n = 6) were made diabetic by injection of alloxan (Sigma Chemical Co., Pool, UK), 65 mg/kg body weight in a single dose into lateral ear vein while they were under anaesthesia (45 mg/kg sodium pentobarbital). All animals were fed ad libitum and standard rabbit plain diet and allowed free access to water.

Induction of diabetes was ascertained by determination of blood glucose concentration in blood obtained from ear artery 72h and weekly after alloxan injection and confirmed by a blood glucose level) 300mg/dL associated with marked reduction in serum insulin level at death.

Measurement of blood glucose concentration and serum insulin level:

Blood glucose was measured by using Reflolux II reflectance photometer (Boehring Mannheim, Germany)

and serum insulin level was determined by Enzyme linked immunosorbant Assay (ELISA) method using specific kits (Bioscience, Europe, SA).

Organ bath studies:

The samples of corpus cavernosal tissue used to study the functional effects of sildenafil were obtained from 5 months diabetic sexually mature male rabbits (n= 6) and age matched control rabbits (n= 6). Under intraperitoneal thiopental anesthesia, penectomies were performed on the animals, with isolation of 2-3 smooth muscle strips (approximately 1x3mm) from the enveloping tunica albuginea of each animal which was then killed by cervical decapitation, the size and weights of the tissues from both control and diabetic rabbits were similar. Freshly dissected cavernosal strips were tied at each end with surgical silk threads and mounted in 10ml capacity water-Jacked tissue bath chambers containing Krebs's-bicarbonate solution (6.9gm NaCl, 2.1 gm NaHCO₃, 0.34 gm KCl, 0.16 gm KH₂PO₄, 0.3 gm MgSO₄, 2.00 gm D.glucose, and 0.18 gm CaCl₂ in 1L distilled water) of pH7.4, maintained at 37°C by thermoregulated circuit and bubbled continuously with 95% oxygen and 5% carbon dioxide.

The upper end of each strip was connected to a lever writing on the rotating drum of the universal kymograph (Bioscience, Washington). The initial resting tension of each strip was set at 2gm, and the tissues were allowed to equilibrate for 60 minutes. The tissue samples were then washed with Krebs solution with a high (120nM) K⁺ concentration (Krebs-KCl solution) (0.22 gm NaCl, 2.10 gm NaHCO₃, 8.86 gm KCl, 0.16 gm KH₂PO₄, 0.30 gm MgSO₄, 1.98 gm D-glucose and 0.18 gm CaCl₂ in 1L distilled water) for 15 minutes to depolarize the tissue and induce a contractile response. After this step, the

tissues were allowed to return to the baseline tension by repeatedly washing them for 30 minutes with Krebs solution. the organ baths were refilled with 10 ml Krebs solution, and then the tissue strips were contracted with phenylephrine hydrochloride at increasing concentrations (10^{-9} – 10^{-3} M) to obtain a dose response curve for the contractile effect of phenylephrine and to find out the submaximal contractile concentration of phenylephrine. In other studies, tissues were:

- a) Precontracted with the submaximal dose of phenylephrine (10^{-5} M) and dose response curves were constructed for the relaxant effect of SNP (10^{-9} – 10^{-3} M), Ach (10^{-9} – 10^{-3} M) and sildenafil (10^{-9} – 10^{-3} M).
- b) Precontracted with phenylephrine (10^{-5} M) and dose response curves were constructed for the relaxant effect of sildenafil (10^{-9} - 10^{-3} M) in the presence of the minimal concentration of either SNP (10^{-8} M) or Ach (10^{-9} M) that induced a significant relaxant effect in order to demonstrate any synergistic or additive effects of sildenafil.
- c) Precontracted with phenylephrine (10^{-5} M), a submaximal dose of sildenafil (10^{-6} M) was then added to the organ bath and after 15min, dose response curves were again constructed for SNP.
- d) Bathed with krebs solution which also contained guanithidine (5×10^{-6} M), atropine (10^{-5} M) and indomethacine (10^{-6} M) to inhibit the adrenergic, cholinergic and cyclooxygenase pathways, respectively leaving the NANC pathway intact. The strip were then, precontracted with phenylephrine (10^{-5} M) and stimulated at frequencies of 4, 8, 12, 16 and 20 Hz, with a stimulator delivering single square waves (duration 0.8 sec) in absence and

presence of sildenafil (10^{-6} M) which was added to the organ bath 15 minutes before electric field stimulations (EFS). These experiments were used to demonstrate the effect of sildenafil on NANC-mediated relaxation of corpus cavernosal strips taken from 5 months diabetic and age matched control rabbits.

Drugs & Chemicals:

Ach, phenylephrine, SNP, alloxan, guanithidine, atropine, and indomethacine were purchased from Sigma Chemical Co. (UK) while sildenafil citrate was obtained from Pfizer Chemical Co. (Egypt).

Statistical analysis:

All data were expressed as mean \pm S.E and statistically analysed according to ANOVA test and student's unpaired to test. A P value < 0.05 was considered statistically significant.

Results

Table (1) illustrates the effects of alloxan-induced diabetes in adult male rabbits. Induction of diabetes significantly increased blood glucose level from 7.02 ± 0.19 mmol/L in normal control group to 22.5 ± 1.78 mmol/L in the diabetic group ($P < 0.001$), and reduced blood insulin level from 82.66 ± 2.4 pmol/L in the control group to reach 27.5 ± 1.74 pmol/L in the diabetic group ($P < 0.001$). Diabetic rabbits also gained significantly less body weight (3.3 ± 0.18 kg) when compared to both their initial body weight [$(3.15 \pm 0.19$ kg), $P > 0.05$] and the final body weight of normal control group [$(4.05 \pm 0.14$ kg), $P < 0.01$].

Table (2), Fig. (1) demonstrate the relaxant effect of sildenafil (in absence and presence of either ACh (10^{-9} M/L) or SNP (10^{-8} M/L), Ach and SNP on phenylephrine (10^{-5} M)-induced contra -

ction in rabbit corpus cavernosal tissue strips. It was found that the inhibitory effect of test agents were ranked (from least inhibitory to most inhibitory) as follows: sildenafil, acetylcholine plus sildenafil, sodium nitroprusside, sodium nitroprusside plus sildenafil, acetylcholine. Sildenafil was observed to have little effect on the phenylephrine-induced contraction even at the highest concentration tested. However, its effect was potentiated in the presence of acetylcholine and SNP.

Table (3) & Fig (2) show the effect of sildenafil on SNP-mediated relaxation of corpus cavernosal strips taken from 5 months diabetic and age-matched control rabbits. The strips were precontracted with phenylephrine (10^{-5} M) in absence and presence of sildenafil (10^{-6} M) which was added 15min before the addition of SNP. Results were expressed as % of relaxation ($\bar{X} \pm S.E$) of the phenylephrine-induced tone.

It was found that SNP-mediated relaxation of 5 months-diabetic corpus cavernosal strips were significantly impaired when compared to age-matched control strips ($P < 0.001$; ANOVA test). A significant difference in SNP-mediated relaxation between age-matched controls and 5 months diabetic cavernosal strips at each data point using student's unpaired t-test, was also observed. On the other hand, it

was noticed that sildenafil significantly enhanced the SNP-mediated corpus cavernosal relaxation in both diabetic and age matched control strips ($P < 0.001$; ANOVA test respectively). A significant difference in SNP-mediated relaxation between the absence and presence of sildenafil at each data point using student's unpaired t-test was also found.

Table (4) & Fig. (3) illustrate the effect of sildenafil (10^{-6} M) on EFS (NANC)-mediated relaxation of corpus cavernosal strips taken from 5 months diabetic and age-matched control rabbits. The strips were precontracted with phenylephrine (10^{-5} M), sildenafil was then added and after 15min, EFS was at frequencies of 4, 8, 12, 16 and 20Hz. It was found that, EFS-induced relaxations were significantly impaired when comparing 5 months diabetic to age-matched control strips ($P < 0.001$ ANOVA test). However, sildenafil significantly enhanced the EFS-stimulated corpus cavernosal relaxations in 5 months diabetic and age-matched control rabbits ($P < 0.001$ & $P < 0.001$, respectively; ANOVA test). A significant difference in EFS-stimulated relaxation between the absence and presence of sildenafil, at each data point, in 5 months diabetic and age-matched control rabbits using a student's unpaired t-test, was also detected.

The Effect of The Specific Phosphodiesterase

Table (1): The effect of alloxan induced diabetes on blood glucose level, serum insulin concentration and body weight in adult male rabbits.

Parameter	Control N=6	Diabetic N=6	P
Blood glucose (mmol/L) $\bar{X} \pm SE$	7.02±0.19	22.5±1.78	<0.001
Blood insulin (pmol/L) $\bar{X} \pm SE$	82.66±2.4	27.5±1.74	<0.001
Initial body weight (Kg) $\bar{X} \pm SE$	3.0±0.15	3.15±0.19	>0.05
Final body weight (Kg) $\bar{X} \pm SE$	4.05±0.14	3.3±0.18	<0.01

Table (2): The effect of SNP, Ach and S [in the presence and absence of either Ach (10^{-9} M) or SNP(10^{-8} M)] on corpus cavernosal strips taken from control rabbits. The strips were precontracted with phenylephrine (10^{-5} M).

DOSES (Mol/L)	S N=6	Ach+S N=6	SNP N=6	SNP+S N=6	Ach N=6	F
10^{-9} $\bar{X} \pm SE$	0	6.66±2.4	8.88±1.4	11.1±1.4	19.9±1.7	20.575**
10^{-8} $\bar{X} \pm SE$	0	12.2±0.99	15.6±1.4	28.8±2.8	35.6±2.2	51.841**
10^{-7} $\bar{X} \pm SE$	3.33±1.5	21.1±3.2	32.2±2.7	46.7±1.7	63.3±2.9	86.903**
10^{-6} $\bar{X} \pm SE$	8.9±2.2	27.8±3.2	43.33±1.5	63.3±2.9	81.1±4.00	97.755**
10^{-5} $\bar{X} \pm SE$	17.7±1.4	36.7±2.85	59.99±3.4	72.2±3.2	92.2±2.04	117.943**
10^{-4} $\bar{X} \pm SE$	23.3±2.3	53.33±2.4	73.33±3.8	84.4±2.2	99.9±2.4	123.710**
10^{-3} $\bar{X} \pm SE$	23.3±2.3	53.33±2.4	73.33±3.8	84.4±2.2	99.9±2.4	123.710**

** p < 0.001

Table (3):The effect of SNP (10^{-9} - 10^{-3} M) on phenylephrine (10^{-5} M)-induced tone of corpus cavernosal strips taken from control and five months diabetic rabbits in the presence of Sildenafil (10^{-6} M).

% of relaxation						
SNP (Mol/L)	Control N=6	Control+S N=6	Control N=6	Diabetic N=6	Diabetic+S N=6	F
10^{-9}	8.88±1.4	18.9±2.04	8.88±1.4	0	5.6±2.09	23.847**
$\bar{X} \pm SE$	<0.01		<0.001			
P	<0.05					
10^{-8}	15.55±1.4	27.8±3.18	15.55±1.4	6.8±2.49	14.6±2.03	13.407**
$\bar{X} \pm SE$	<0.01		<0.05			
P	<0.05					
10^{-7}	32.2±2.7	44.4±2.2	32.2±2.7	15.7±2.2	28.8±1.4	29.577**
$\bar{X} \pm SE$	<0.01		<0.001			
P	<0.001					
10^{-6}	43.33±1.5	58.9±2.05	43.33±1.5	25.6±3.13	38.9±2.7	32.153**
$\bar{X} \pm SE$	<0.001		<0.001			
P	<0.01					
10^{-5}	59.99±3.4	74.33±2.1	59.99±3.4	39.99±3.4	52.2±2.1	25.789**
$\bar{X} \pm SE$	<0.01		<0.01			
P	<0.05					
10^{-4}	73.33±3.8	88.8±2.2	59.99±3.4	54.4±2.04	69.99±3.8	20.994**
$\bar{X} \pm SE$	<0.01		<0.01			
P	<0.01					
10^{-3}	73.33±3.8	88.8±2.2	59.99±3.4	54.4±2.04	69.99±3.8	20.994**
$\bar{X} \pm SE$	<0.01		<0.01			
P	<0.01					

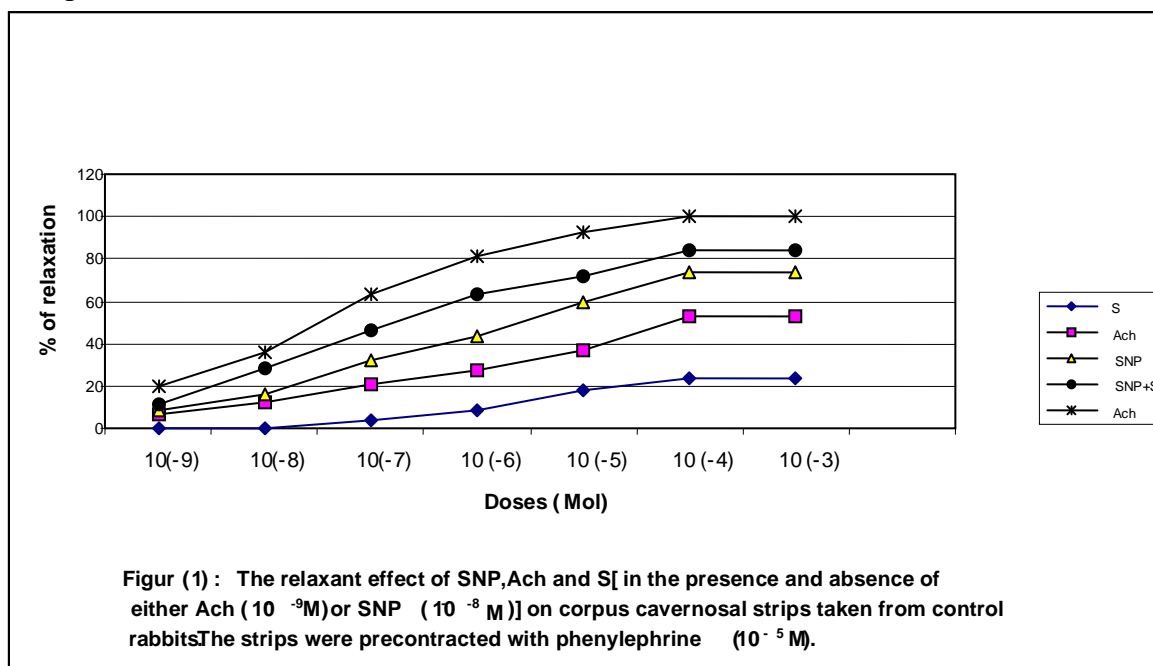
** p< 0.001

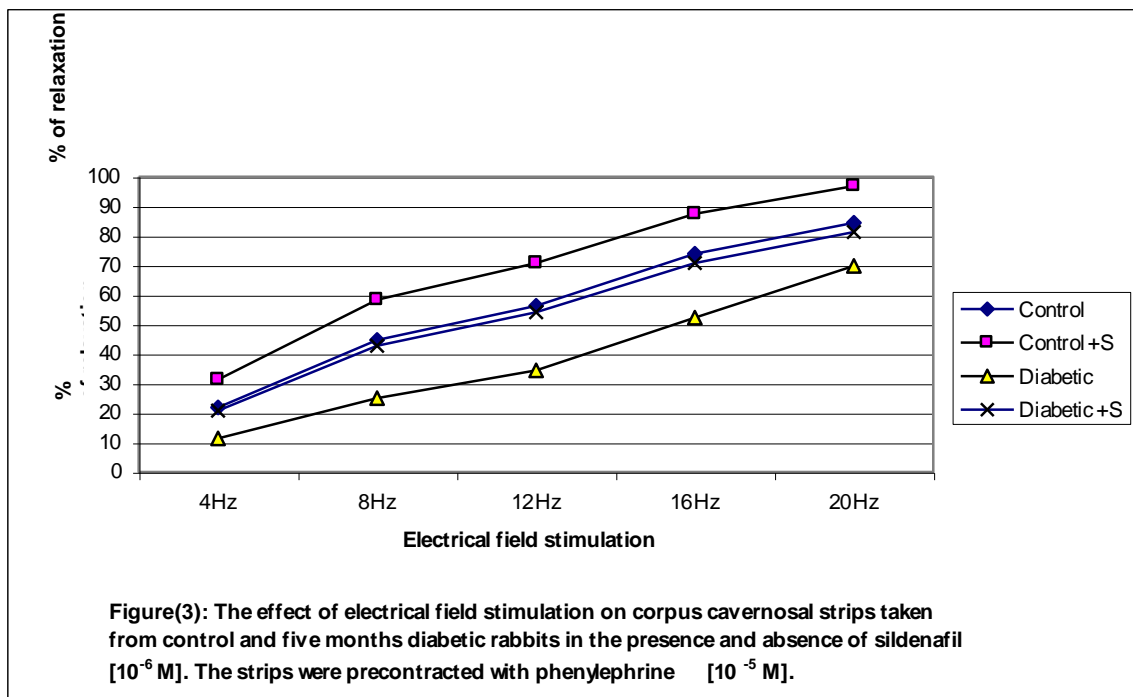
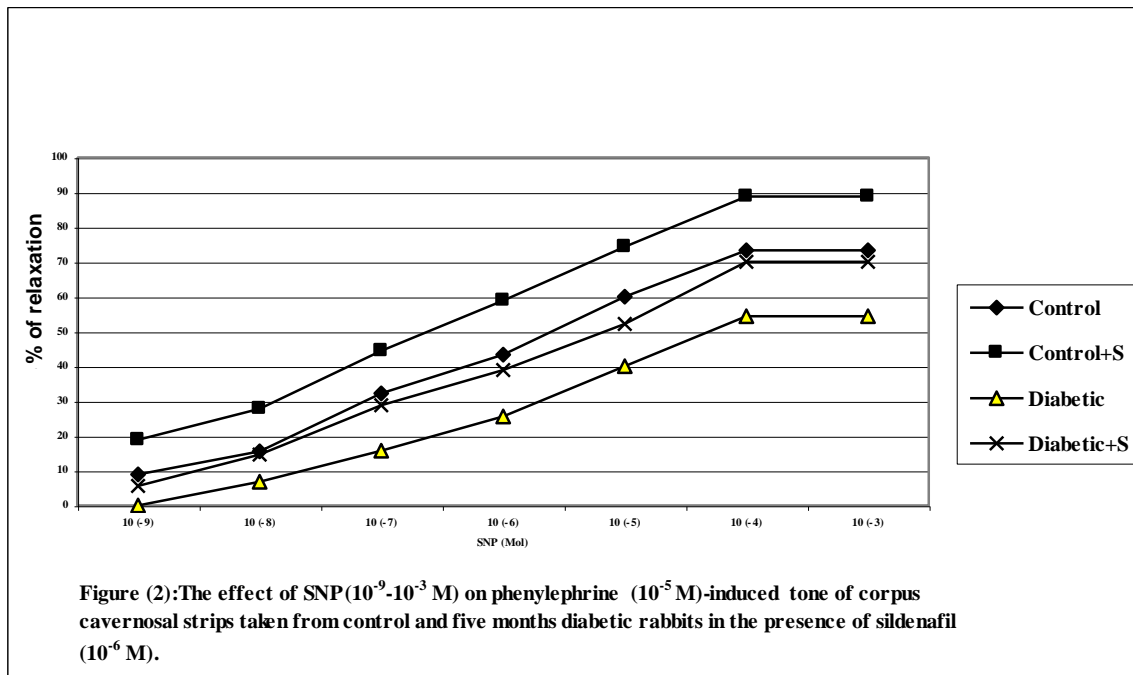
The Effect of The Specific Phosphodiesterase

Table (4):The relaxant effect of electrical field stimulation on corpus cavernosal strips taken from control and five months diabetic rabbits in the presence and absence of sildenafil (10^{-6} M). The strips were precontracted with phenylephrine (10^{-5} M).

% of relaxation						
EFS	Control N=6	Control+S N=6	Control N=6	Diabetic N=6	Diabetic+S N=6	F
4Hz						
$\bar{X} \pm SE$	21.87±2.7	31.25±2.3	21.87±2.7	11.37±1.9	20.8±2.02	13.097**
P	<0.05		<0.01			
						<0.01
8Hz						
$\bar{X} \pm SE$	44.7±3.4	58.33±2.1	44.7±3.4	25±2.28	42.7±1.9	21.993**
P	<0.01		<0.001			
						<0.001
12Hz						
$\bar{X} \pm SE$	56.3±3.2	70.8±3.09	56.3±3.2	34.37±2.7	54.16±3.1	24.493**
P	<0.01		<0.001			
						<0.001
16Hz						
$\bar{X} \pm SE$	73.95±2.9	87.5±2.28	73.95±2.9	52.28±2.6	70.8±2.08	23.547**
P	<0.01		<0.001			
						<0.001
20Hz						
$\bar{X} \pm SE$	84.4±2.7	96.87±1.4	84.4±2.7	69.8±3.02	81.25±2.3	21.168**
P	<0.01		<0.01			
						<0.05

**p< 0.001





Discussion

It has been well established that corpus cavernosal smooth muscle relaxation required for erectile function is mainly dependent on the neuronal NO-cGMP system (Burnet, 1995; Carter et al., 1998). Many studies have shown that the potentiating effects of NO synthase inhibitors and NO blockers on erectile

mechanisms, both in vitro and in vivo (Trigo-Rocha et al., 1993; 1994 & Wang et al., 1993).

At present much evidence supports the concept that NO derives from the autonomic innervation of the penis and locally operates as a postganglionic neurotransmitter of non-adrenergic,

non-cholinergic-mediated penile erection. On its synthesis and release from the nerve terminals within the erectile tissue of the penis, NO diffuses into the adjacent smooth muscle cells of the penis. There, it activates the soluble guanylate cyclase present in smooth muscle cells to produce cGMP, which results in smooth muscle relaxation (Ignarro *et al.*, 1990; Bush *et al.*, 1992; Burnett, 1997). Recent research suggests that NO activates Na⁺-K⁺-ATPase in human and rabbit corporeal smooth muscle musculature, as well as the potassium conductive membrane hyperpolarization pathway in rabbit corporeal smooth muscle cells (Burnett, 1997).

The present study revealed that sildenafil had little effect on the phenylephrine-induced contraction and this effect was potentiated, at least partially, in the presence of acetylcholine. Sodium nitroprusside (SNP) which breaks down spontaneously in aqueous solution to generate NO have a more relaxant effect on corpus cavernosal strips precontracted with phenylephrine than that of sildenafil alone or sildenafil in the presence of acetylcholine. These results are in agreement with that of Aydin *et al.* (2001).

The dilatation of vascular beds by choline esters is due to the presence of muscarinic receptors, primarily of M₃ subtype, despite the lack of apparent cholinergic innervation of most blood vessels. The muscarinic receptors responsible for relaxation are located on endothelial cells of the vasculature; when these receptors are stimulated, the endothelial cells release the endothelium-derived relaxing factor, or NO, which diffuses to adjacent smooth muscle cells and causes them to relax (Brown and Taylor, 1995). Thus, NO release from NANC neurons is not the only mechanism involved in erection.

This indicates that the principle mode of action of sildenafil is through enhancement of cAMP accumulation, which is dependent not only on a prior endogenous NANC stimuli but also on the parasympathetic drive acting through NO. Therefore, the relaxing action of sildenafil is at least partially mediated by endothelium.

This proposal, in a way, describes why sildenafil treatment improves erectile function in patients with erectile dysfunction secondary to traumatic spinal cord injury, with no difference in patients who did and did not have residual erectile function at baseline (Hultling *et al.*, 1998). Clinical reports about improved erectile function after radical prostatectomy (Lowentritt *et al.*, 1999 & Zippe *et al.*, 1998) can also be considered within this concept. However, other biochemical substances locally released from the endothelial or smooth muscle components of the erectile tissue may also take part in this function. The premise that NO could modulate corporeal smooth muscle function concerns with its original description as an endothelium-derived relaxing factor, this substance was discovered in vasculature to derive from endothelial cells and to induce vascular smooth muscle relaxation (Palmer *et al.*, 1987). Cholinergic agents, alpha-adrenergic antagonists, endothelium-derived relaxing factor, peptides, prostaglandins, histamine, calcium channel blocking agents, and other nonspecific vasodilators are some of the substances that induce cavernosal muscle relaxation (Anderson, 1993 & Holmquist *et al.*, 1990).

However, the present study showed that acetylcholine alone when used at high concentrations was a more effective relaxant agent than all other treatment agents including the combination of sodium nitroprusside

and sildenafil. This contradictory result implies that although acetylcholine releases NO by stimulating muscarinic receptors at lower concentrations, high concentrations of acetylcholine has a direct relaxation effect on isolated rabbit cavernosal smooth muscle. The relaxation effect of acetylcholine at higher concentrations may be mediated by decreasing the intracellular sequestration, probably by direct reduction of calcium transport by way of voltage-dependent calcium channels or sarcoplasmic reticulum not by hyperpolarization or NO. Extensive studies are needed for clarification.

The data of the present study indicate that sildenafil enhances the relaxing effect of both SNP and Ach on phenylephrine-induced contractions of rabbit cavernosal tissue in varying degrees. However, functional organ both studies must be interpreted with caution. Because the cyclic nucleotide turnover rates in isolated tissue preparations are low, relatively high concentrations of phosphodiesterase inhibitors are needed to elevate the cyclic nucleotide levels and produce a significant tissue response. Additionally, the effects of sildenafil on rabbit corporeal smooth muscle may not be entirely translatable to humans, even though the phosphodiesterase isoenzyme distribution may be similar homogeneously.

The present study revealed that SNP-stimulated corpus cavernosal smooth muscle relaxation was impaired in diabetic rabbits compared to age matched controls. This impairment was enhanced in the presence of sildenafil which was observed to enhance SNP-stimulated relaxation of corpus cavernosal tissue from age-matched control rabbits. These findings are in accordance with those of Ballard *et al.* (1996) and Rendell *et al.* (1999) who showed that sildenafil potentiates NO-mediated

corpus cavernosal relaxation of healthy rabbits and reported that sildenafil enhances cGMP formation and accumulation. Our findings are also in agreement with that of Thompson *et al.* (2001) and Utkan *et al.* (2001) who showed that SNP-stimulated cGMP formation was impaired in diabetic rabbit corpus cavernosal tissue and it was improved in the presence of sildenafil. These studies together with our study provide evidence for the possibility of using selective phosphodiesterase inhibitors in the treatment of diabetic impotence.

Since SNP 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). These differences may be ascribed to species variation and 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 DeTejada, 1991).

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). As sildenafil normalized the reduced SNP-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 and demonstrate that type V phosphodiesterase activity is intact in diabetic corpus cavernosal tissue.

Thompson *et al.* (2001) found that SNP & Ach-stimulated cGMP formation was impaired in the diabetic group compared to age-matched controls and observed that this impairment was overcome in the presence of sildenafil. As stimulation by ACh is dependent on NO release by the activation of endothelial NO-synthase (e NOS) leading to endothelium-dependent NO-mediated smooth muscle relaxation, there could also be a defect in eNOS activity in diabetics apart from impaired guanylyl cyclase activity as previously suggested by Sullivan *et al.* (1998) who also showed that SNP-stimulated cGMP formation was not significantly different between age-matched controls and diabetic corpus cavernosal tissues.

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). One of the principal neurotransmitters released by NANC neurons is NO, which causes the formation of cGMP and corpus cavernosal relaxation (Bredt and Snyder, 1994).

In the present study, a significant impairment in electrical field stimulation-mediated corpus cavernosal relaxation after 5 months of diabetes mellitus was found. This result is in line with that of Thompson *et al.* (2001). Since electrical field stimulation elicits relaxation through activation of the NANC neurons that innervate the corpus cavernosum, these data suggest that diabetic neuropathy may have developed after 5 months of diabetes mellitus. There is evidence to support this supposition, since early signs of

vascular neuropathy characterized 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 this 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 was found to enhance the relaxation of the corpus cavernosum in response to electrical field stimulation in age-matched controls and 5 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 can not 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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تأثير السيلدينافيل ستريت المثبط النوعي لإنزيم الفوسفوداي إستيريز 5 على عضلات الجسم الكهفي الملساء للأرنب المصاب بمرض السكر المحدث بمادة الألوكسان

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لقد أجريت هذه الدراسة لفحص:

تأثير كل من عقار السيلدينافيل المثبط لعمل إنزيم الفوسفوداي إستيريز 5 و نيتروبروسيد الصوديوم الذي يؤدي إلى زيادة أكسيد النيتريك دون الاعتماد على الغشاء المبطن للجسم الكهفي والأسيتيل كولين الذي يؤدي إلى زيادة أكسيد النيتريك اعتمادا على الغشاء المبطن للجسم الكهفي بطريقة منفردة ومجمعة على شرائح الجسم الكهفي المعزولة والمنقبضة بتأثير مادة الفينيل إفرين.

التأثيرات الغير أدرينيرجيكية والغير كولينيرجيكية والتي تتم من خلال أكسيد النيتريك على شرائح الجسم الكهفي المعزولة للأرنب المصاب بمرض البول السكري لمدة خمسة شهور والمحدث تجريبيا عن طريق الحقن الوريدي لمادة الألوكسان بجرعة قدرها 65 مجم/كجم وقد سبق انقباض هذه العضلات الملساء بمادة الفينيل إفرين.

تأثير المجال الكهربائي على شرائح الجسم الكهفي المعزولة من الأرنب المصاب بمرض البول السكري والمنقبضة بمادة الفينيل إفرين وذلك في وجود وغياب عقار السيلدينافيل.

وقد أظهرت الدراسة أن كلا من الأسيتيل كولين و نيتروبروسيد الصوديوم والسيلدينافيل ذو تأثير انبساطي على العضلات الملساء للجسم الكهفي المعزول والمنقبضة بمادة الفينيل إفرين وأن تأثير الأسيتيل كولين منفردا كان أقوى من تأثير نيتروبروسيد الصوديوم في وجود السيلدينافيل يلي ذلك تأثير نيتروبروسيد الصوديوم منفردا ثم تأثير الأسيتيل كولين في وجود السيلدينافيل ثم تأثير السيلدينافيل منفردا والذي يعتبر أقلهم تثبيطا للانقباض العضلي المحدث بتأثير الفينيل إفرين.

أما بالنسبة للمجموعة المصابة بمرض البول السكري فقد أوضحت الدراسة أن:

التأثير الانبساطي لمادة نيتروبروسيد الصوديوم على عضلات الجسم الكهفي الملساء المعزولة المنقبضة بمادة الفينيل إفرين أقل بدرجة كبيرة ذات دلالة إحصائية مقارنة بالمجموعة السليمة الضابطة وقد تحسن هذا التأثير بدرجة ذات دلالة إحصائية في وجود عقار السيلدينافيل.

هناك نقصا ذو دلالة إحصائية في الاستجابة الانبساطية لعضلات الجسم الكهفي المعزولة المنقبضة بمادة الفينيل إفرين تحت تأثير المجال الكهربائي مقارنة بالمجموعة السليمة الضابطة ولكن بإضافة عقار السيلدينافيل لوحظ أن هناك تحسنا ذو دلالة إحصائية في هذه الاستجابة الانبساطية.

وهكذا فإن هذه الدراسة تخلص إلى أن عقار السيلدينافيل المثبط النوعي لعمل إنزيم الفوسفوداي إستيريز 5 والذي يؤدي بدوره إلى زيادة تركيز جوانوزين أحادي الفوسفات يلعب دورا حيويا وهاما في علاج الخلل الوظيفي لعضلات الجسم الكهفي الملساء في حالات الإصابة بمرض البول السكري الذي يحدث نقصا في تركيز أكسيد النيتريك المفرز من كل من الأعصاب الغير أدرينيرجيكية والغير كولينيرجيكية المغذية للجسم الكهفي وكذلك من الغشاء المبطن للجسم الكهفي.