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The Effect of Sildenafil Citrate (Viagra) Combination with Vitamin E on Some Blood Neurotransmitters and Minerals in Diabetic Rats

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Abstract: Erectile Dysfunction (ED) is a prevalent problem among most of diabetic patients. This defect is extensively treated by Sildenafil Citrate (SC), which is a potent phosphodiesterase -5 inhibitor (PDE-5 I). The mechanism of SC in treating ED in an experimental diabetic model, in conjunction with a potent antioxidant, vitamin E (vit E) is to be elucidated. Here, we report that, whether alone or in combination with E, SC induces penile erection, mostly, through organized modulation of blood neurotransmitters, namely, Serotonin (Ser), Dopamine (Dop), Nor-Epinephrine (NEP) and Nitric Oxide (NO), in addition to potassium (K), controlled calcium (Ca) influx modulation, affecting neurotransmitters' release into the circulation, without apparent impact on sodium level (Na). In the present study, Ser, Dop, NEP and NO blood levels were together, significantly up-regulated in normo-glycemic and more significantly in alloxan diabetic rats by SC treatment, an effect which was non-significantly prohibited by E combination. K level was elevated by SC in normo-glycemic, but decreased in diabetic rats. This later action was reversed by E, however, Na level was only significantly elevated in diabetic group, given SC combined with E, but Ca levels were non-significantly changed. These actions of SC elucidate-in part-the mechanism of its action and necessitate care to its impact on diseases related to autonomic neurotransmission.

Key words: Sildenafil, serotonin, dopamine, nor-epinephrine, nitric oxide, calcium, sodium, potassium, diabetes, rats

INTRODUCTION

Erectile Dysfunction (ED) is considered as a life morbidifying disorder that always does not announce itself until it becomes a terrible threat for the couple. This illness is probably a more complicating cause of life in a broad sector of peoples specially, Middle Eastern, due to many social, financial and religious considerations. The treatment of ED has changed significantly in the past few years. Revolutionary advances in oral treatment have replaced invasive modalities in many cases (Shabsigh and Anastasiadis, 2003). Sildenafil citrate tablets became one of the most-if not the most-prescribed medicine world wide, with or without prescription (McCullough, 2002).

In Egypt, it is now the most common Over The Counter (OTC) drug. It was reported that this molecule, primarily known as UK-92-480, showed marked coronary artery dilatation when introduced for first clinical trials. In these primary trials, the patients noticed that the drug showed an erectogenic effect (Boolell *et al.*, 1996). This adverse action opened new area for the use of the drug and extensive studies were conducted for the study of its mechanism of action. It is now well established that the drug competitively inhibits phosphodiesterase, type 5 (PDE 5) enzyme activity, due to the great analogy of both molecular structures (Uckert *et al.*, 2000).

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The inhibition of PDE 5 activity increases the available cyclic Guanosine Mono-Phosphate (cGMP) in the penile and corpus cavernosal muscles, leading to relaxation of these muscles and increased blood flow, which promptly initiates erection (Dey and Shepherd, 2002). This pathway is completely accomplished through the release of NO, which is the principal neurotransmitter, controlling, with, biogenic amines, the penile vascular dilatation (Grasgasin *et al.*, 2004).

However, penile smooth muscle activity requires several factors, including adequate local levels of neurotransmitters, adequate expression of receptors, integrity of transduction mechanisms and ion channel homeostasis (de Tajeda *et al.*, 2000).

Thus, in addition to NO, nor-epinephrine (NEP), dopamine (Dop) and serotonin (Ser) contribute-together-to the sexual functions and they are always the targets for drugs affecting ED (Meston and Frochlich, 2000).

Most of metabolic diseases are predisposing factors for ED, like hypertension and diabetes (Kalsi and Kell, 2004). Diabetes represents the major risk factor for ED (Spollett, 1999). Thus, among men with ED, 15% are diabetic and 50% of diabetics will develop some degree of ED after 5 years of being diabetic. The underlying causes may be poor glycemic control, progressive diabetic vasculopathy, neuropathy, myopathy as well as underlying depression. Most of these cases demonstrated considerable response to SC treatment (Price *et al.*, 2004).

It is clear that ED is mostly governed by neurotransmitters that have direct action on corpus cavernosum. However, neurotransmitter release depends mostly on signals initiated through calcium-gated potassium channels (Steers, 2002).

These findings will be developed in our study, which was exclusively initiated to justify and/or secure the reported mortalities about cardiovascular and diabetic patients, in a trial to contribute to future therapeutic strategies for these susceptible sectors in society. As well as, to outline most neurotransmitters pertinent to sexual activity, in addition to some minerals necessary for neurotransmitter exocytosis, in an experimental model of diabetes, treated with SC oral doses. These neurotransmitters included, Ser, Dop, NEP and NO, in addition to Na, K and Ca in blood samples of alloxan-diabetic rats. Vitamin E (vit E) was also tried as an anti-oxidant to assess whether it would be of value in modulating the drug efficacy in both normo-glycemic and diabetic animals.

MATERIALS AND METHODS

Animals

Sixty four male Wistar rats weighing 100-120 g were purchased from the animal house of the college of medicine, Assiut University, Egypt, around December 2004. The animals were kept in polyethylene cages of 60×40×30 cm dimensions at temperature range of 15-20°C, fairly humid room in 12 h light/12 h dark adjusted conditions. Rats were fed standard rat chow and allowed to drink normal tap water ad libitum and left 10 days to acclimatize before dosing started. They were classified into 8 equal groups and assigned from 1 to 8 groups. Group 1 was left as control and have not given any medication. Group 2 was given SC (formal chemical name (IUPAC): 1-[4-ethoxy-3-(6,7-dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl) phenylsulfo-nyl]-4-methylpiperazine, Pfizer, USA), orally, as single dose of 3 mg kg⁻¹ body weight by gavage (Baratti and Boccia, 1999).

Group 3 was given vit E orally, as 300 mg kg⁻¹ (Farco, Egypt), daily (Fu and Liu, 1992). Group 4 was given both SC and E, 30 minutes apart, in the same doses on a daily basis. The first four groups were considered as normo-glycemic. The animals of group 5 were given 150 mg kg⁻¹ alloxan, intraperitoneally, blood glucose was measured daily, by blood puncture, the dose could be repeated till blood glucose level reached about 270 mg dL⁻¹. This level was considered as the diabetic level (Kisel *et al.*, 2001). This group served as diabetic control. The rest 3 group were got diabetic using the same alloxan dosing system, then, the sixth group was given SC doses as group 2, while group 7 was

given SC as group 3 and group 8 was given SC plus E as group 4. The animals of groups 5-8 were considered diabetic animals. All animals were killed after 2 weeks of dosing. Fasting blood samples were withdrawn, centrifuged, sera were divided into aliquots and kept frozen at -80°C right analysis.

Methods

The level of Ser, Dop and NEP was fluorometrically estimated according to the method of Schlumpf *et al.* (1974). NO was determined colorimetrically, based on the enzymatic conversion of nitrate to nitrite by nitrate reductase, forming a chromophoric azo-derivative through Griess reaction (Miles *et al.*, 1996). Sodium level was determined photometrically (Usovich *et al.*, 1975). Potassium was also photometrically determined (Hillmann and Beyer, 1967). Calcium level was colorimetrically estimated using O-Cresolphthalein complexone (Stern and Lewis, 1957). Data were analyzed by one way ANOVA and differences were calculated using Duncan's new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

As shown in Table 1, SC significantly up-regulated blood levels of Ser, Dop, NEP and NO in all treated subjects. Combination of SC and E significantly elevated these parameters, except NO in normo-glycemic rats, which was not changed, however, E alone, non-significantly elevated both Ser and NEP, but significantly elevated both Dop and NO in normo-glycemic rats. In diabetic rats, SC whether alone or combined with E, significantly elevated the studied neurotransmitters.

In Table 2, SC showed non-significant effects on both Na and Ca, but significantly elevated K levels in normo-glycemic rats. It significantly decreased K level in diabetic group. Addition of E to SC, only elevated Na level to a significant extent ($p < 0.05$). E alone, non-significantly affected Na, K and Ca levels in all groups.

Erection is the result of a complex series of integrated neuronal and vascular events that lead to accumulation of blood in the penis to achieve rigidity. The coordination of several neural events is required to release endogenous neurotransmitters at the level of penile smooth muscle to induce relaxation of penile vasculature which eventually and promptly triggers erection. Disruption of this series of events can lead to ED (Andersson and Wagner, 1995; Moreland *et al.*, 2000). Our finding on

Table 1: Effect of sildenafil citrate (3 mg kg⁻¹ body weight) combination with vitamin E (300 mg kg⁻¹) on serotonin, dopamine, norepinephrine and nitric oxide blood levels in normo-glycemic and diabetic rats after two weeks of oral administration

Parameters	Normo-glycemic				Diabetic			
	Control	SC	Vit. E	SC+Vit. E	Control	SC	Vit. E	SC+Vit. E
SER ($\mu\text{g mL}^{-1}$)	0.84±0.02	1.1±0.06**	0.91±0.01	1.20±0.07**	0.84±0.01	0.98±0.04**	0.93±0.02	1.2±0.04**
Dop ($\mu\text{g mL}^{-1}$)	0.96±0.03	1.2±0.02**	1.10±0.02**	1.30±0.02**	0.96±0.01	1.20±0.07**	1.10±0.03*	1.3±0.02**
NEP ($\mu\text{g mL}^{-1}$)	3.50±0.14	4.8±0.23**	3.80±0.15	4.90±0.08**	4.00±0.21	5.70±0.22**	4.10±0.29	5.7±0.12**
NO ($\mu\text{mol L}^{-1}$)	7.20±0.54	9.4±0.29**	8.80±0.20**	7.23±0.21	15.40±0.70	56.00±2.70**	29.60±1.10**	22.8±0.44**

*: Significantly different from control at $p < 0.05$, **: Significantly different from control at $p < 0.01$; Values are expressed in Mean±SE (n = 8)

Table 2 Effect of sildenafil citrate (3 mg kg⁻¹ body weight) combination with vitamin vit E (300 mg kg⁻¹) on sodium, potassium and calcium blood levels in normo-glycemic and diabetic rats after two weeks of oral administration

Parameters	Normo-glycemic				Diabetic			
	Control	SC	Vit. E	SC+Vit. E	Control	SC	Vit. E	SC+Vit. E
Na (mmol L^{-1})	164.8±7.70	164.9±2.80	149.0±3.60	173.7±5.40	156.3±2.40	170.8±5.20	152.2±3.40	174.5±6.90*
K (mmol L^{-1})	3.2±0.20	4.7±0.30**	3.3±0.20	3.3±0.30	3.7±0.20	2.5±0.20**	3.8±0.20	3.0±0.20
Ca (mg dL^{-1})	10.7±0.34	10.5±0.21	10.9±0.24	10.9±0.23	9.9±0.43	10.3±0.45	10.5±0.25	9.4±0.43

*: Significantly different from control at $p < 0.05$, **: Significantly different from control at $p < 0.01$; Values are expressed in Mean±SE (n = 8)

Ser showed an accordance to that reported about SC which improved co-morbid ED and mild to major depression (Rosen *et al.*, 2006). This increased availability of Ser was suggested to be valuable in the treatment of psychogenic ED (Nehra *et al.*, 1999).

Moreover, Ser availability may contribute-in part-to correcting diabetes-induced penile vascular and neural derangements responsible for ED (Giuliano *et al.*, 1999). Nevertheless, it seems that the role Ser in ED is still conflicting, because it exerts its effect on sexual function through two counteracting receptors i.e., serotonin 1A receptor up-regulation is useful in reversing sexual dysfunction (Landen *et al.*, 1999), although another receptor (serotonin₂) may be down-regulated to maintain the same serotonin effect (Eison *et al.*, 1990). Alternatively, serotonin levels increase in studied group of rats when they become sexually receptive (Luine, 1993).

Thus, study of the effect of SC on both Ser receptors and expression would be a necessary approach. In our results, significant up-regulation of Dop and NEP blood levels was interpreted through reports saying that penile erection is the end result of smooth muscle relaxation, which can be initiated by sensory stimulation that activates central nervous system pathways (de Groat and Booth, 1993). These processes activate penile peripheral nerves, which include cholinergic, noradrenergic, non cholinergic (NO), Vasoactive Intestinal Peptide (VIP) and potentially calcitonin gene-related peptide containing nerves entering the pelvic plexus from sacral (S₂-S₄) region. This complex activation increases oxygen tension in addition to increased local prostaglandin synthesis, leading to efficient erection (Kim *et al.*, 1993). In the present work, SC or SC+ vit E induced Dop up-regulation is considered as a contributory element in initiation of erection through dopamine receptor agonism (Morales *et al.*, 1995). This can be explained by experimental evidence showing that dopaminergic mechanisms are involved in determining libido and causing penile erection (Heaton, 2000). This was also confirmed by clinical studies handling ED in parkinsonian patients treated with SC (Hussain *et al.*, 2001). It could be attributed to Dop activation of D₂ receptors, as activation of D₁ receptors by Dop inhibits sexual activity (Pomerantz, 1991). This effect of Dop on D₂ receptor was confirmed by its action as an inducer for erection that was antagonized by haloperidol (D₂ antagonist and antipsychotic), (Gower *et al.*, 1984), but it was potentiated by D₂ agonists (apomorphine, LY 163502 and RDS-127) (Clark *et al.*, 1983; Foreman and Hall, 1987; Hull *et al.*, 1986), respectively.

Elevated NEP blood levels were positively correlated with sexual activity and erection (Wiedeking *et al.*, 1979; Kruger *et al.*, 1998). Given that vanillylmandelic acid is the metabolite of NEP, it was reported that both NEP and its metabolite were elevated in sexual activity (Ende *et al.*, 1989). SC whether given alone or in combination with E, similarly E alone, significantly elevated NO blood levels in both normo-glycemic and diabetic rats except in normo-glycemic group which given SC+ vit E combination (Table 1). NO has been the subject of intense interest since its biological description by Ignarro *et al.* (1987). It is now understood to be the most significant mediator of vascular smooth muscle relaxation, responsible for engorgement of erectile tissue in men (Bivalacqua *et al.*, 2001). NO was considered the main neurotransmitter mediating erectile function, which is released from the endothelium of corpora cavernosa during non-adrenergic, non-cholinergic neurotransmission (Rajfer *et al.*, 1992). Our results, showed an agreement with that reported by Kloner (2001), who reported that SC enhanced the relaxant effect of NO released into the corpora cavernosa from non-adrenergic, non-cholinergic autonomic nerves and vascular endothelium during sexual stimulation. In the present work, circulating NO was significantly elevated in diabetic subjects given either SC, vit E or SC+ vit E combination. This result is in accordance with the published results of De Young *et al.* (2004).

It could be attributed to the preserving action of E to endothelial vasomotor tissues (Kinlay *et al.*, 1999). In our work, Na level was not significantly changed in all groups, except the diabetic group that

given SC+ vit E, it was significantly increased than diabetic control. SC treatment significantly elevated K level in normo-glycemic, but decreased it in diabetic subjects. Ca levels were non-significantly changed (Table 2). Studies made on Na levels under the effect of SC are very sparse. The effect of SC on both K, NO and Ca levels coincides with the work of Lue (2000), who registered that, within the muscle of corpus cavernosum, NO activates guanylyl cyclase, which raises the intracellular concentration of cyclic Guanosine Mono Phosphate (cGMP). In turn, it opens K channels and sequester intracellular Ca by endoplasmic reticulum resulting in dilation of arterial vessels and increasing the blood of low into the sinuses of the corpora cavernosa. Thus any event that limits Ca entry to the cell or release from intracellular storage will ultimately have significant impact on corporal smooth muscle tone (Stief *et al.*, 1997). Moreover, SC was reported to directly, relax cavernous muscle through receptor-operated inhibition of Ca influx into cavernous tissue (Lau and Adaikan, 2006).

Up to 50% of men with diabetes mellitus have ED (Ledda, 2000). Endothelium dependent relaxation of cavernous smooth muscle is highly reduced in diabetic men with ED (de Tejada *et al.*, 1989). This was attributed to impaired endothelial function in hyperglycemia patients (Moody *et al.*, 1997) and diabetic animal models (Giuliano *et al.*, 1999). The role of vit E in increasing K, NO-but decreasing, non-significantly Ca level-contributing to initiation of erection in diabetic rats treated by SC, was supported by the work of De young *et al.* (2003) who reported that supplementation with vit E (a free radical scavenger) increased phosphodiesterase inhibitor, type 5 (PDE5)-mediated erection in diabetic rats.

In conclusion, SC mostly initiates erection through up-regulation of endothelium relaxant factor (NO), simultaneously with Ser, Dop and NEP blood levels. This effect was potentiated by vit E supplementation, prominently in alloxan diabetic rats. The drug did not show experimentally significant effects on Na, K and Ca levels in normo-glycemic, but non-significantly decreased in diabetic subjects. Taken together, the role of the drug in correcting ED is more efficient when E is supplemented. This antioxidant addition will be much more recommended for diabetic patients using sildenafil citrate, which may be useful in combating many cardiovascular and diabetic adverse drug effects.

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