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ORIGINAL ARTICLE

Comparative effects of sildenafil, phentolamine, yohimbine and L-arginine on the rabbit corpus cavernosum

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ABSTRACT

Penile erection involves relaxation of smooth muscle of corpus cavernosum and associated arterioles. Sildenafil, a highly selective inhibitor of phosphodiesterase type 5, is effective in the treatment of erectile dysfunction. The aim of this study is to investigate the effect of sildenafil on smooth muscle of the rabbit corpus cavernosum (RCC) and to compare its effect with those of phentolamine, yohimbine and L-arginine. The effects of sildenafil, phentolamine, yohimbine and L-arginine were studied on the response of the RCC to electrical field stimulation (EFS) as well as on the phenylephrine (PE, 3×10^{-6} M)-induced tone. EFS caused transient, frequency-dependent relaxation of the RCC that was inhibited by the nitric oxide synthase inhibitor N^{G}-nitro-L-arginine (3×10^{-5} M). Sildenafil (1×10^{-9}–1×10^{-6} M) and phentolamine (1×10^{-9}–1×10^{-6} M) enhanced the EFS-induced relaxation in a concentration-dependent manner with ED_{50} of 0.056 ± 0.004 and 0.572 ± 0.035 μM at 8 Hz, respectively, yohimbine (3×10^{-7}–3×10^{-5} M) and L-arginine (3×10^{-6}–3×10^{-4} M) did not show significant effects (ED_{50} at 8 Hz = 35.84 ± 2.24 and 2.164 ± 0.174 μM, respectively). Sildenafil (1×10^{-9} and 1×10^{-8} M) potentiated the EFS-induced relaxation caused by L-arginine (3×10^{-5} M). Sildenafil, phentolamine, yohimbine and L-arginine reduced the PE-induced tone to different extents; the ED_{50} values were 0.81 ± 0.097, 0.49 ± 0.025 and 13.97 ± 1.10 μM, respectively. Maximum concentration of L-arginine used failed to produce 50% relaxation ($ED_{20} = 221.82 \pm 15.71$ μM). The muscle relaxant effects of different combinations of sildenafil and L-arginine on PE-induced tone did not differ significantly from the sum of the individual effects. The results demonstrate that sildenafil, when compared to other drugs used in penile erection dysfunction, shows the highest potency on the nitrergic transmission in the RCC. On the other hand, phentolamine was found to possess the highest potency in inducing relaxation of RCC proving that its action is independent on the stimulated neurogenic system. In addition, the combination of less effective doses of sildenafil and L-arginine has a potential advantage on erectile functions. The importance of this combination remains to be solved clinically.

INTRODUCTION

Penile erection is a hemodynamic process involving relaxation of smooth muscle of corpus cavernosum and its associated arterioles. The pivotal role of nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) in the mediation of the erectile process have been studied [1]. It has become increasingly clear that a NO–cGMP inhibitory system plays a predominant role in mediating penile erection [2,3]. During sexual stimulation smooth muscle relaxation is mediated by NO release from nerve terminals within the erectile tissue of the

penis. NO diffuses to the neighboring vascular and trabecular smooth musculature whereby it binds to the heme component of soluble guanylate cyclase present in smooth muscle cells stimulating synthesis of cGMP. Binding of cGMP to cGMP-dependent protein kinase can alter the contractile state of the smooth muscle [2,4].

Current strategies for the pharmacological treatment of erectile dysfunction (ED) favor the use of oral agents, including the phosphodiesterase type 5 (PDE5) inhibitor sildenafil, as well as phentolamine, yohimbine, L-arginine and others [5]. Sildenafil was found to be effective in improving erectile function and enabling successful sexual intercourse in men with broad spectrum ED (organic, physiological or mixed origin) [6]. It has been claimed that previous production of cGMP by NO [7] is required for sildenafil activity. Thus, sildenafil promotes penile erection only in response to sexual stimulation which is responsible for the production of NO from nonadrenergic noncholinergic (NANC) cavernosal nerves. Oral phentolamine (a non-selective antagonist for α-adrenoceptors), was found to play a critical role in initiating and/or maintaining penile erection [8]. It has been proposed to improve erections in patients with psychogenic and mild arteriogenic ED. Phentolamine was reported to exhibit erectogenic properties both by α-blocking effect on the corpus cavernosum as well as through a central anti-anxiety effect [9]. Yohimbine is an indole alkaloid that has been widely used for treatment of psychogenic impotence [10,11]. Yohimbine's aphrodisiac activity may be mediated through a combination of central and peripheral blockade of pre- and postsynaptic α_2-adrenergic receptors [12]. NO, which is believed to be the major neurotransmitter involved in erection, is derived from the amino acid L-arginine. Zorgniotti and Lizza [13] found that oral administration of L-arginine twice daily caused an improved erectile function in 40% of patients with psychogenic or vascular impotence.

This study aims to compare the potency of sildenafil with that of phentolamine, yohimbine and L-arginine using the rabbit corpus cavernosum (RCC) smooth muscle. Two experimental protocols representing penile erection were used to determine the relative potencies of drugs under test. A part of this study had the objective to determine the effect of L-arginine on sildenafil-induced relaxation of RCC, in order to evaluate the possible potential use of drug combination for the treatment of ED.

MATERIALS AND METHODS

Tension studies

Thirty-five adult, sexually mature, male New Zealand white rabbits weighing 2.5–3 kg were used. Animals were obtained from Alexandria Faculty of Pharmacy Animal House. Water and food were allowed ad libitum. Rabbits were killed and their penises were excised rapidly and placed in Krebs solution (composition, mM: NaCl 118, KCl 4.6, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.6, $CaCl_2 \cdot 2H_2O$ 1.2, $NaHCO_3$ 24.0, glucose 11) at 4 °C. The corpora were dissected and subsequently studied in organ chambers [14]. From each rabbit two to four strips were obtained.

Tissue baths containing Krebs solution were kept at 37 °C and constantly bubbled with 95% O_2 and 5% CO_2. One end of each strip was attached to a fixed pin at the bottom of the organ bath. The other end was attached to a force displacement transducer (Grass FT-03), which was connected to a Grass polygraph (Model 7D). The initial resting tension was 2 ***g***. The preparation was left to equilibrate for 90 min. Four to five tension experiments are done on the same strip.

In electric field stimulation experiments, each strip was submaximally contracted with 3×10^{-6} M phenylephrine (PE), and subjected to electrical field stimulation (EFS)-induced relaxation at supramaximal voltage (0.8 ms pulse duration) using sequential frequencies of 2, 4, 8, 16 and 32 Hz delivered as 10-s trains. Stimulation was provided with two parallel platinum electrodes 4–5 mm apart surrounding the middle portion of the strip. These electrodes were attached to a Grass electronic stimulator (Model S 48). The bathing media routinely contained atropine (1×10^{-6} M) and guanethidine (5×10^{-6} M) to block muscarinic receptors and prevent the release of norepinephrine, respectively, during subsequent EFS. After wash, the strips were incubated with the chosen drug (sildenafil, phentolamine, yohimbine, L-arginine or a combination of sildenafil and L-arginine) for 15 min before being again contracted with PE and EFS being repeated. When the effect of NO-synthase inhibitor N^G-nitro-L-arginine (L-NNA) (3×10^{-5} M) was examined, it was added to the strips, and after 10 min, EFS was repeated. Responses to different treatments were assessed by determining the level of tissue relaxation at 2–32 Hz. The tissue relaxation responses by EFS were assessed as a percentage of the PE-induced contractile response; if tone decreased below baseline, a relaxation response greater than 100% was calculated. In some experiments, where incubation

with a specific concentration of a drug caused more than 30% inhibition of PE-induced tone, the concentration of PE was adjusted to produce a similar level of tone for each experimental condition.

In PE-induced tonic experiments, each muscle was submaximally contracted with PE (3×10^{-6} M). After the PE contractile response has stabilized, relaxation responses to different treatments were recorded in a cumulative fashion. For the characterization of sildenafil-induced muscle relaxation, contraction with PE and relaxation responses were obtained following incubation with L-NNA for 10 min.

The relaxation responses were expressed as a percentage of submaximal contraction produced by PE [15]. Control organ chamber was similarly contracted with the same concentration of PE; however, appropriate concentrations of the drug vehicle were added.

Statistics

Results were expressed as mean ± SEM. Student's *t*-test was used for the analysis of unpaired data. For multiple comparison (i.e. more than one mean), the one-way analysis of variance (ANOVA or *F* test) followed by Student–Newman–Keuls post test was utilized. The criterion for statistical significance was set at the 0.05 level. The potencies of different drugs were expressed as ED_{50}. ED_{50} values were determined by regression analysis of the linear portion (approximately 15–85% range) of the concentration response curve in individual tissues. Statistical analysis is performed using a computer software program GRAPH PAD INSTAT (version 2.01) copy right © 1990–1993, Steve Whetzel, Parke-Davis 930762 A.

Drugs used

The following drugs were used: sildenafil (Pfizer, New York, USA), atropine sulfate, guanethidine, phentolamine, phenylephrine HCl, yohimbine, L-arginine, N^G-nitro-L-arginine (Sigma Chemicals Co., St Louis, MO). All drugs were dissolved in distilled water.

RESULTS

Characterization of EFS-induced relaxation of RCC

Strips of RCC showed contractile responses to the addition of 3×10^{-6} M PE. The concentration of PE applied caused about 80% of maximal PE-inducible tension. Further application of EFS (0.8-ms duration, for 10 s at a supramaximal voltage) elicited transient, frequency-dependent relaxation responses that increased in magnitude over the range of 2–32 Hz. Relaxation of RCC in response to EFS was markedly inhibited by L-NNA (3×10^{-5} M) (*Figure 1* and *Table I*).

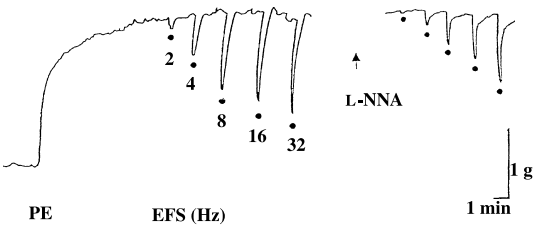

Figure 1 Representative tracing showing the effect of N^G-nitro-L-arginine (L-NNA, 3×10^{-5} M) on nonadrenergic noncholinergic relaxations induced by electrical field stimulation (EFS, 2–32 Hz, 0.8-ms duration, supramaximal voltage) on phenylephrine (PE, 3×10^{-6} M)-precontracted strips of rabbit corpus cavernosum.

Table I Effect of N^G-nitro-L-arginine (3×10^{-5} M) on nonadrenergic noncholinergic relaxations induced by electrical field stimulation on phenylephrine (3×10^{-6} M)-precontracted strips of rabbit corpus cavernosum.

	Percentage relaxation	
Frequency (Hz)	Before	After
2	5.64 ± 1.89 (7)	0.00 ± 0.00 (7)*
4	25.47 ± 7.27 (7)	7.74 ± 2.98 (7)*
8	47.59 ± 11.71 (7)	15.06 ± 2.98 (7)*
16	52.12 ± 8.08 (6)	27.83 ± 5.05 (6)*
32	62.62 ± 7.82 (6)	37.23 ± 6.10 (6)*

Responses are expressed as mean ± SEM. Values between parentheses indicate the number of observations. *Denotes significant difference from control at the level of $P < 0.05$.

Effects of sildenafil, phentolamine, yohimbine and L-arginine on the amplitude of EFS-induced relaxation of the RCC

In the presence of adrenergic and cholinergic receptors blockade, sildenafil (1×10^{-9}–1×10^{-6} M) and phentolamine (1×10^{-9}–1×10^{-6} M) enhanced the EFS-induced relaxation responses of RCC in a concentration-dependent manner. Sildenafil at concentrations $\geq 1 \times 10^{-7}$ M produced significant augmentation of relaxations than control at all frequencies (*Figures 2* and 3), while phentolamine produced significant augmentation of relaxation only at a concentration of 1×10^{-6} M. Although L-arginine (3×10^{-6}–3×10^{-4} M) increased the amplitude of NANC relaxations, no significant potentiation was obtained. Similarly, no significant effect on EFS-induced relaxations was

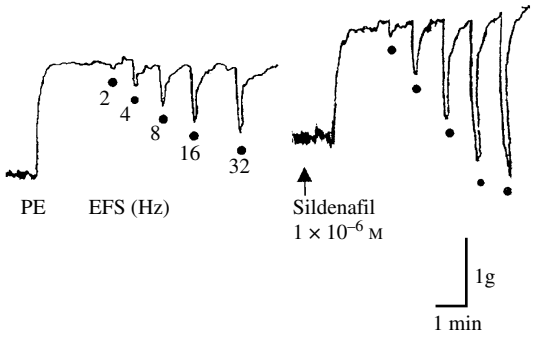

Figure 2 Representative tracing showing the effect of sildenafil (1×10^{-6} M) on the amplitude of nonadrenergic noncholinergic relaxations induced by EFS (2–32 Hz, 0.8-ms duration, supramaximal voltage) on PE-precontracted strips of rabbit corpus cavernosum.

observed for yohimbine, the effect induced by yohimbine was greater at lower concentrations. For comparison of potency of selected drugs in inducing augmentation of NANC responses at 8 Hz, calculated ED_{50} for sildenafil, phentolamine, yohimbine and L-arginine were 0.056 ± 0.004, 0.572 ± 0.035, 35.84 ± 2.24 and 2.164 ± 0.174 μM, respectively (*Figure 4*).

Effects of combination of sildenafil and L-arginine on the amplitude of EFS-induced relaxation of RCC

Different combinations of sildenafil (1×10^{-9}–1×10^{-7} μM) and L-arginine (3×10^{-6}–3×10^{-5} M) were tested for their effects on NANC relaxations and compared to the effect of the corresponding single dose of either drug. Taking the intermediate frequency (8 Hz) as a model for comparison, percentage potentiation of NANC relaxations induced by combinations was greater than the sum of effects of individual drugs (the corresponding concentrations of either drug had no significant effect. *Figure* 5 shows that the effect of L-arginine (3×10^{-5} M) in combinations was more pronounced than the effect of the lower dose.

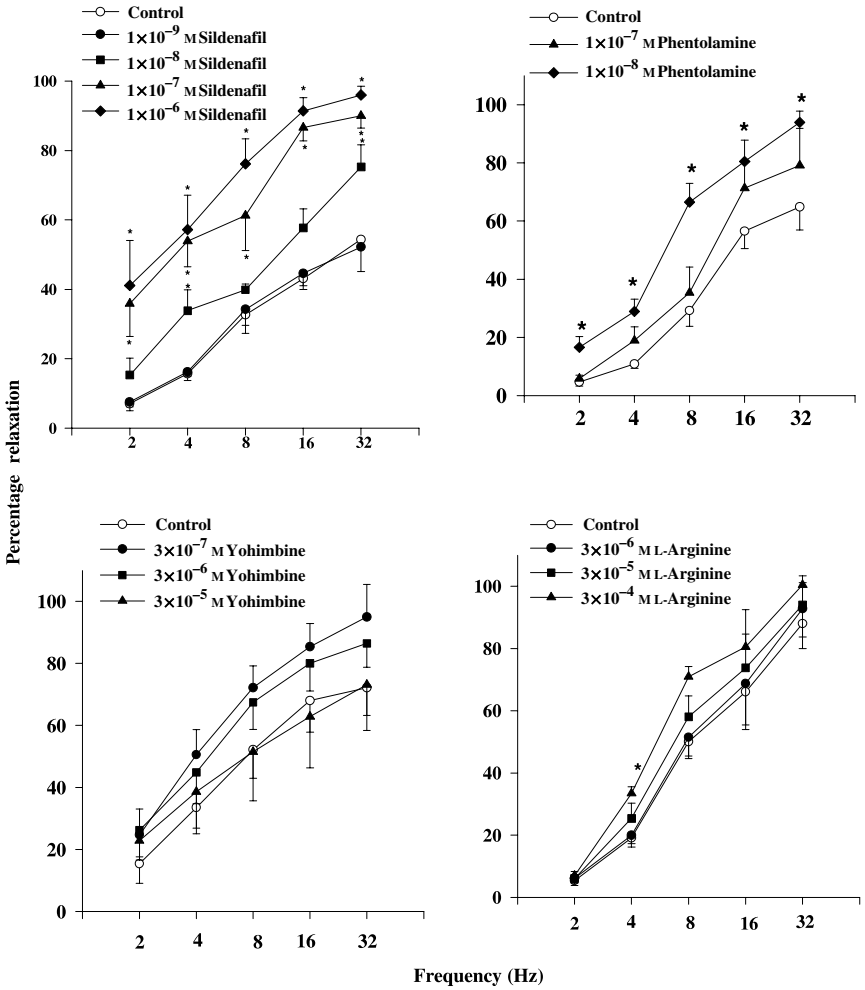

Figure 3 Effects of sildenafil (1×10^{-9}–1×10^{-6} M), phentolamine (1×10^{-7}–1×10^{-6} M), yohimbine (3×10^{-7}–3×10^{-5} M) and L-arginine (3×10^{-6}–3×10^{-4} M) on the amplitude of nonadrenergic noncholinergic relaxations induced by EFS on PE-precontracted strips of rabbit corpus cavernosum. Responses are expressed as mean ± SEM of 10–15 observations for control groups and five to eight observations for treated groups. *Denotes significant difference from control at the level of $P < 0.05$.

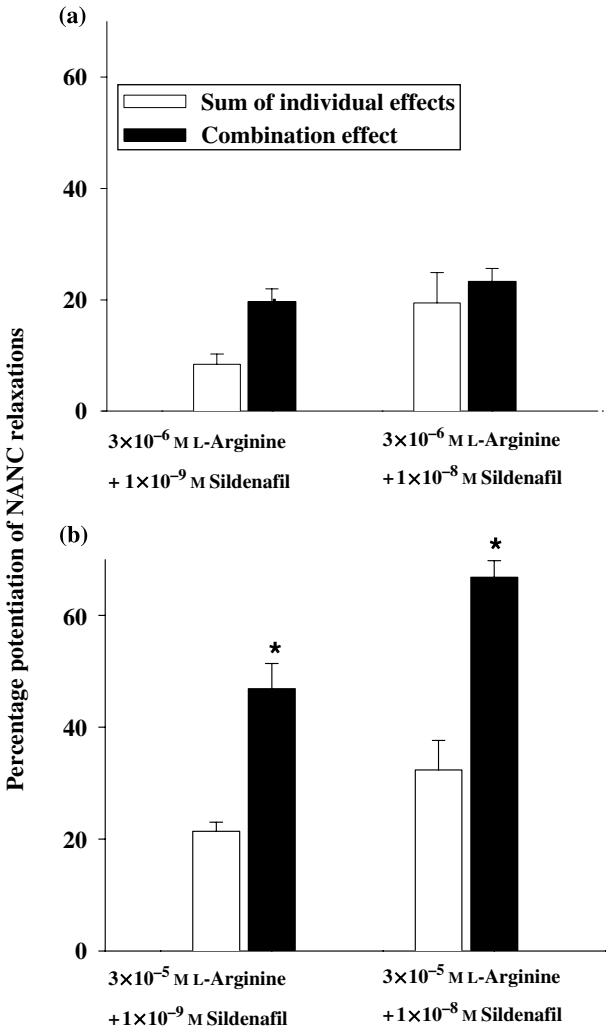

Figure 4 Effects of combination of sildenafil (1×10^{-9} and 1×10^{-8} M) and L-arginine (3×10^{-6} M, histogram a and 3×10^{-5} M, histogram b) on the potentiation of the amplitude of nonadrenergic noncholinergic relaxations induced by EFS at 8 Hz on PE-precontracted strips of rabbit corpus cavernosum. Responses are expressed as mean ± SEM of five to six observations. *Denotes significant difference from the sum of individual effects at the level of $P < 0.05$.

Relaxant effects of sildenafil, phentolamine, yohimbine and L-arginine on phenylephrine-precontracted strips of RCC

The addition of PE (3×10^{-6} M) to the bathing solution resulted in smooth muscle contraction that rapidly attained a steady state of increased tension. The addition of cumulative doses of sildenafil (1×10^{-9}–1×10^{-6} M), phentolamine (1×10^{-9}–1×10^{-6} M), yohimbine (3×10^{-7}–3×10^{-5} M) and L-arginine (3×10^{-6}–3×10^{-4} M) resulted in significant smooth muscle relaxation (*Figure 6*). Yohimbine showed the highest efficacy

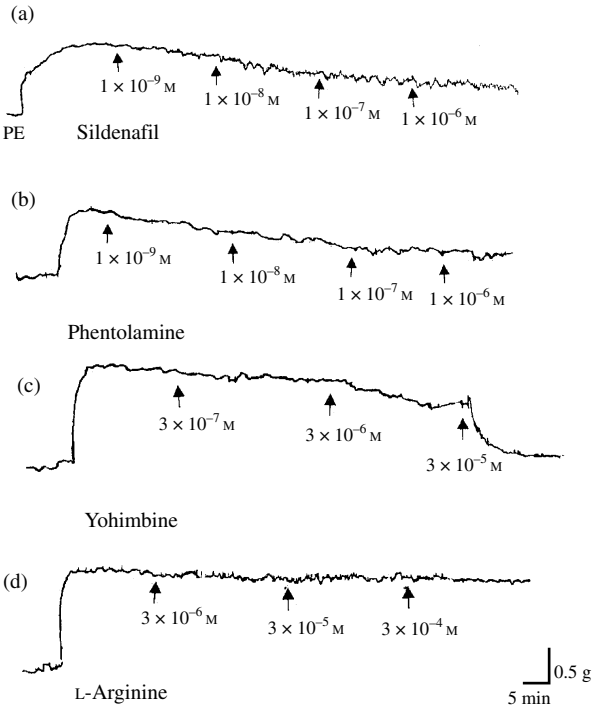

Figure 5 Representative tracings showing the muscle relaxant effects of sildenafil (trace a), phentolamine (trace b), yohimbine (trace c) and L-arginine (trace d) on PE-induced tone of the rabbit corpus cavernosum strips.

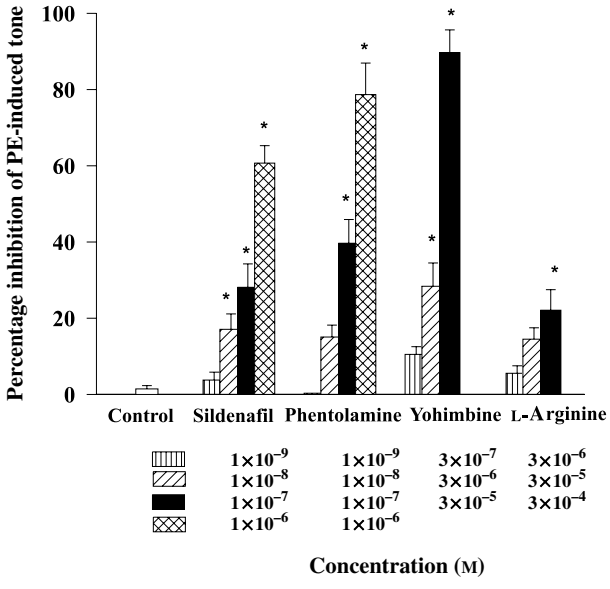

Figure 6 Muscle relaxant effects of sildenafil, phentolamine, yohimbine and L-arginine on PE-induced tone of the rabbit corpus cavernosum strips. Responses are expressed as mean ± SEM of five to six observations. *Denotes significant difference from control at the level of $P < 0.05$.

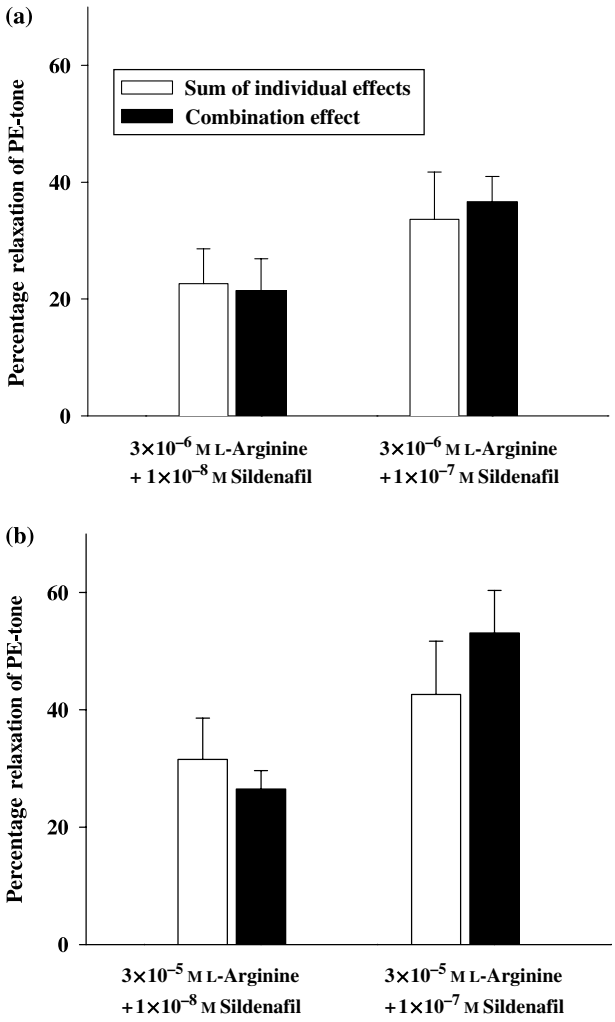

Figure 7 Effects of combination of sildenafil (1×10^{-8} and 1×10^{-7} M) and L-arginine (3×10^{-6} M, histogram a and 3×10^{-5} M, histogram b) on PE-induced tone of the rabbit corpus cavernosum strips. Responses are expressed as mean ± SEM of five to eight observations.

(89.68 ± 5.97%), while phentolamine, which reduced PE-induced tone by 78.66 ± 8.27% showed the highest potency among the drugs tested (*Figure 7*).

Respective ED_{50} for sildenafil, phentolamine and yohimbine were 0.81 ± 0.097, 0.49 ± 0.025 and 13.97 ± 1.10 μM. L-arginine was the least potent drug showing an ED_{20} of 221.82 ± 15.70 μM.

Relaxant effects of combination of sildenafil and L-arginine on phenylephrine-precontracted strips of RCC

Significant reduction of PE-induced tension was observed upon addition of selected combinations of sildenafil and L-arginine to the strips of RCC. When compared to the relaxant effects of the sum of corresponding concentrations of either drug, it was found that 3×10^{-6} M and 3×10^{-5} M L-arginine were able to augment the relaxant effect of only sildenafil 1×10^{-7} M when used in combination. L-arginine 3×10^{-6} M plus sildenafil 1×10^{-7} M, produced 36.66% relaxation of PE-tone compared to 33.66% for the sum, while 3×10^{-5} M plus 1×10^{-7} M for the respective drugs produced 53.08% relaxation compared to 42.59% for the sum (*Figure 7b*). The effects of these combinations did not differ significantly from the sum of the individual effects.

DISCUSSION

The importance of NO in the erectile function is supported by the results obtained with the NO synthase inhibitor L-NNA. EFS-induced relaxation was inhibited significantly by L-NNA (3×10^{-5} M), as shown in previous studies [7,15–17]. The electrically elicited relaxation of corporal smooth muscle was completely blocked by the addition of tetrodotoxin, a neuronal sodium channel blocker [7]. This indicates that electrical stimulation triggered an exclusively neurogenic response [15,18]. This is consistent with the hypothesis that electrically elicited relaxation is mediated by the NANC, NO-dependent neuronal pathway [19–21].

At concentrations of 1×10^{-8} M and above, sildenafil significantly potentiated the neurogenic NO-mediated relaxation of RCC strips induced by EFS in a concentration-dependent manner, and similar results were obtained in strips of human corpus cavernosum [18]. Zaprinast, another PDE5 inhibitor, was reported to be less potent in this respect [7]. It seems that, sildenafil amplifies the neuronal NO–cGMP pathway involved in relaxation of corpus cavernosum smooth muscle. The EFS of PE-precontracted cavernosal strips is selected to investigate the effect of drugs on nitrergic transmission in erectile tissue. Sildenafil was found to be the most potent drug capable of modulating nitrergic transmission in erectile smooth muscle compared to phentolamine, yohimbine and L-arginine. This may support the view that sildenafil does not elicit erection in the absence of sexual arousal in contrast to yohimbine or phentolamine intracavernosal injection [22]. L-arginine, being a NO substrate, was reported to have significant effects on the systemic vascular tone regulated by NO [23]. However, studies of the effect of L-arginine on erectile function are few [13]. The present results showed that L-arginine had no significant effect on EFS-induced

relaxations of PE-precontracted strips of RCC at all frequencies tested (except the higher dose at 8 Hz). Similar results for L-arginine have also been observed in other studies [24–26]. It seems that L-arginine is ineffective in single dose therapy, but long-term treatment with L-arginine would provide better improvement of erectile functions [27].

Despite the modest effect of L-arginine on neurogenic relaxations, it was able to augment the effect of sildenafil on EFS-induced relaxations. L-arginine at the dose of 3×10^{-5} M increased this potentiatory effect of sildenafil at concentrations of 1×10^{-9} and 1×10^{-8} M at 8 Hz. The magnitude of NANC relaxations in the presence of these combinations was higher than the sum of the corresponding effects of sildenafil and L-arginine. These results give rise to the potential advantage of the combination of sildenafil and L-arginine on erectile function. The fact that the concomitant use of sildenafil and nitrovasodilators is contraindicated [28] does not contradict the possible potential use of sildenafil and L-arginine in combination, since it might replenish the stores of NO by L-arginine; therefore raising the possibility of reducing the dose of sildenafil when combined with L-arginine.

The PE-precontracted RCC strip represents a model that does not depend on NO–cGMP-induced relaxation. The second protocol was designed to closely mimic the adrenergically induced tone that maintains the penis in the flaccid state [29]. It was earlier reported that suppression of adrenergic tone, together with direct cavernosal smooth muscle relaxation is needed to induce penile erection [30]. Among the drugs tested, phentolamine was the most potent smooth muscle relaxant drug followed by sildenafil and yohimbine, with respective ED_{50} of 0.49 ± 0.025, 0.81 ± 0.10 and 13.97 ± 1.10 μM. L-arginine failed to produce 50% relaxation (ED_{20} = 221.92 ± 15.70 μM). This may explain the possible effectiveness of yohimbine and phentolamine in ED in absence of sexual arousal. This also raises the assumption that sildenafil may act at least partly by the relaxation of the cavernosal smoth muscle. Such mechanism is proved to be independent of an originally stimulated neurogenic system as confirmed by a previous study [8]. Using isolated human cavernous smooth muscle sildenafil had a superior effect compared to rolipram (selective PDE4-inhibitor) and zaprinast [31].

L-arginine (3×10^{-6} and 3×10^{-5} M) produced potentiation of sildenafil (1×10^{-8} and 1×10^{-7} M)-induced muscle relaxation. The effect of L-arginine in combination with sildenafil was less significant on the smooth muscle relaxation protocol than the EFS one, indicating that these combinations may have a potential advantage mainly on the modulation of nitrergic transmission. It can be concluded that these combination may facilitate erection depending only on an originally stimulated neurogenic system (i.e. in the presence of sexual arousal).

CONCLUSION

The results demonstrate that sildenafil, when compared to other drugs used in penile erection dysfunction, exerts the highest potency on the nitrergic transmission using the RCC. On the other hand, phentolamine was more potent in inducing relaxation of RCC, which indicates that its action is mainly independent of an originally stimulated neurogenic system. In addition, the use of lower effective doses of sildenafil in combination with L-arginine may present a potential advantage in the treatment of ED. The importance of this combination remains to be solved clinically.

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