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EFFECT OF THE SELECTIVE PHOSPHODIESTERASE TYPE 5 INHIBITOR SILDENAFIL ON ERECTILE FUNCTION IN THE ANESTHETIZED DOG

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ABSTRACT

Purpose: The effects of sildenafil, a highly selective inhibitor of cyclic guanosine monophosphate-specific phosphodiesterase type 5, on erectile function in the anesthetized dog were evaluated.

Materials and Methods: In pentobarbital-anesthetized dogs, increases in intracavernosal pressure in the corpus cavernosum and penile blood flow were induced by pelvic nerve stimulation over a frequency range of 1 to 16 hertz. The effects of increasing doses of sildenafil on electrically stimulated intracavernosal pressure, penile blood flow, blood pressure, and heart-rate were evaluated. In parallel experiments, the effects of the nitric oxide synthase inhibitor Nω-Nitro-L-Arginine (L-NOArg) on these same parameters also were assessed.

Results: The effects of nerve stimulation on intracavernosal pressure and blood flow to the penis were blocked by L-NOArg, 0.1–3 mg./kg., in a dose-related manner, confirming the important role of nitric oxide in producing erections. Sildenafil, 1–100 μg./kg administered intravenously, had no direct effect on intracavernosal pressure but potentiated the increase in intracavernosal pressure induced by nerve stimulation. This potentiation occurred at sildenafil plasma concentrations consistent with its relaxation effect on isolated human cavernosal tissue and its inhibition of phosphodiesterase type 5 in vitro. Sildenafil had no significant effect on blood pressure or heart rate.

Conclusions: By inhibiting cyclic guanosine monophosphate-specific phosphodiesterase type 5, sildenafil augments the neuronal mechanism responsible for penile erection. This mechanism explains the significant improvements reported in the rigidity and duration of erections seen in patients with erectile dysfunction who have been treated with oral sildenafil.

KEY WORDS: penile erection, male, dog, 3',5'-cyclic-GMP phosphodiesterase inhibitors, impotence

Erectile dysfunction (ED) is a common medical condition[1,2] for which there is no highly effective oral treatment.[3,4] ED can be classified as either psychogenic or organic, depending on its etiology, but frequently a psychogenic component exists in the etiology of organic ED.[4] Management of this disease relies on a range of treatments, such as psychotherapy, self-injection or transurethral application of vasodilator agents, vacuum constriction devices, prosthesis implantation, and venous/arterial surgery.[4]

The physiology and pharmacology of penile erection have been widely investigated over the past several years. Many studies have highlighted the pivotal role of nitric oxide (NO) and its stimulation of cyclic guanosine monophosphate (cGMP) in the mediation of the erectile process.[5] Sildenafil is a selective phosphodiesterase type 5 (PDE5) inhibitor,[6] which is being clinically evaluated for the oral treatment of both psychogenic and organic ED. In early clinical trials, sildenafil has significantly improved erectile function in patients with ED.[6,7] Fig. 1 shows a schematic diagram of the NO/cGMP pathway of penile erection and the effect of sildenafil on this pathway. These mechanisms have been studied in rabbits,[8–10] dogs,[11–13] and monkeys.[14] Most of these animal studies have used similar methods for the measurement of intracavernosal pressure (ICP). However, the dog probably offers the best model for the measurement of a wide range of other complementary hemodynamic parameters, such as blood pressure, heart rate, regional blood flow, and vascular resistance.

A dog model has been used in which increases in ICP and pudendal artery blood flow are produced by electrical stimulation of the cavernosal branch of the pelvic nerve.

This paper describes this model and demonstrates its reproducibility and stability in control animals. The mechanisms involved in penile erection are assessed in experiments using the nitric oxide synthase inhibitor *Nω*-nitro-L-arginine (L-NOArg). Finally, the effects of sildenafil were evaluated in this dog model of erectile function.

MATERIALS AND METHODS

Male beagles, with body weights of 12–14 kg., were deprived of food overnight. Animals were anesthetized with sodium pentobarbital, 30–45 mg./kg., administered intravenously via an angiocatheter in the brachial vein. Anesthesia was maintained throughout the experiments by a continual infusion of pentobarbital 1–1.3 ml./hr. A catheter was introduced into the left femoral vein for the administration of compounds. The left femoral artery was cannulated for the measurement of blood pressure, and a lead II electrocardiogram was recorded to determine heart rate. Both ureters were cannulated via a mid-line abdominal incision to prevent urine accumulation in the bladder, and the bladder was completely emptied. The left internal pudendal artery was carefully dissected free of surrounding tissues to allow a flow probe to be placed around it. Blood flow was measured using a Transonic flow meter (Transonic Instruments Inc., Ithaca,

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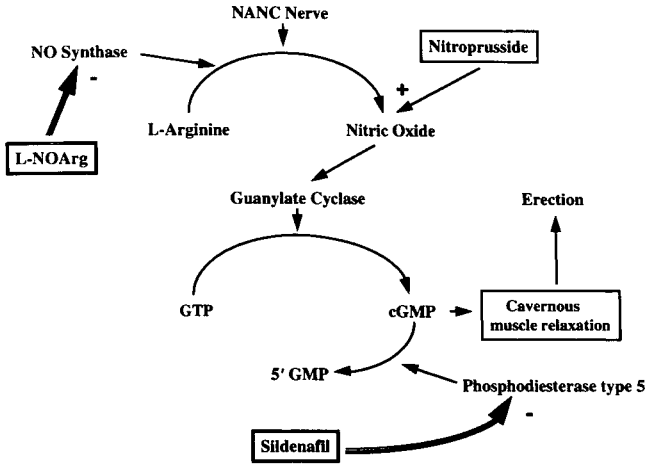

FIG. 1. Cyclic GMP pathway: Nitric oxide (NO) is released from L-arginine following stimulation of pelvic nerve (NANC). This can be inhibited by Nω-nitro-L-arginine (L-NOArg). NO can also be released from an NO donor, such as sodium nitroprusside. NO diffuses into smooth muscle cells activating soluble guanylate cyclase, which catalyzes breakdown of GTP to cGMP, which in turn stimulates smooth muscle relaxation. cGMP is converted to GMP by the enzyme phosphodiesterase. Sildenafil selectively inhibits phosphodiesterase type 5, thereby enhancing action of cGMP. Minus sign denotes inhibition and plus sign denotes stimulation. Adapted from Trigo-Rocha, F. et al.[12]

NY). The cavernosal branch of the pelvic nerve was identified and a small section was dissected free and placed into bipolar stimulating electrodes. The penis was carefully denuded of skin down to the base, without damaging the prepuce, and the left corpus cavernosum was exposed. A 21-gauge scalp needle, attached by flexible catheter to a pressure transducer, was inserted into the corpus cavernosum for the measurement of intracavernosal pressure (ICP). The system was filled with heparinized saline (15–20 U/ml.), which was flushed through prior to each measurement cycle. The dogs were artificially respired with a Ugo Basile 5025 dog ventilator (Ugo Basile, Varese, Italy), adjusted to maintain blood gasses at pO_2 95–115 mm. Hg; pCO_2 25–40 mm. Hg; and pH 7.35–7.45. Expired air was continually monitored by a Datex Normocap 200 (Datex Instrumentarium Corp., Helsinki, Finland) to aid respiratory control. Body temperature was maintained at 36–38C using an electric blanket (Harvard Instruments, South Natick, Mass.). Parameters were recorded on a Grass model 7D polygraph (Grass Inc., Quincy, Mass.) and all data acquisition and calculation of derived parameters was carried out on-line using a Motorola 68000-based microcomputer system. The pelvic nerve was stimulated with a Grass S88 stimulator (Grass Inc.) at 10–15 volts, with a 2-millisecond pulse width, which gave optimum responses, for 1 minute to ensure that pressure increases had reached a plateau over a frequency range of 1–16 hertz. Changes in ICP were expressed as percent of mean blood pressure. Blood flow parameters were expressed as percent maximum flow produced by stimulation at 16 hertz.

A series of four experiments were performed with the NO synthase inhibitor *Nω*-nitro-L-arginine (L-NOArg) (Sigma, Poole, United Kingdom) to evaluate the major mechanisms involved in pelvic nerve-stimulated increases in intracavernosal pressure and blood flow. Following a period of equilibration, control frequency-response curves were generated beginning at a frequency of 1 hertz. The peak pressure within the corpus cavernosum and peak penile blood flow were measured. This was repeated at 2, 4, 8, and 16 hertz, when the previous responses had returned to baseline. A second control cycle was started 40 minutes after the first cycle was completed. L-NOArg was dissolved in saline and a 100 μg./kg. dose was administered intravenously, with electrical stimulation commencing 5 minutes post-dose. The dose of L-NOArg was increased at 40-minute intervals to a maximum dose of 3 mg./kg. Drug concentrations of L-NOArg were calculated on the basis of its molar weight/kg. body weight, allowing 'pseudo' pA_2 values to be calculated for both increases in ICP and internal pudendal artery blood flow by Schild analysis using frequency ratios derived from stimulation frequency-response curves. The term 'pseudo' pA_2 was used because the calculation was based on the assumption that the maximum ICP and blood flow increases were equivalent to those produced, under control conditions, by stimulation at 16 hertz.

Experiments with sildenafil were performed in a similar way to those described for L-NOArg. Sildenafil was dissolved in saline and given at doses of 1–100 μg./kg., with stimulation cycles beginning 15 minutes after dosing. In addition, blood samples were drawn just prior to electrical stimulation for measurement of sildenafil plasma concentrations, using the methods described by Boolell et al.[6]

Frequency-response curves obtained in the presence of various doses of sildenafil were compared with the control curve using analysis of variance, based on the assumption that the effect of changing dose was to displace the frequency response curves in a vertical direction. In other analyses, Student's *t* test was used.

RESULTS

Electrical stimulation of the cavernosal nerve produced a rapid increase in internal pudendal artery blood flow, followed, after a brief latent period, by an increase in ICP. These increases were maintained during stimulation and decreased rapidly when stimulation was stopped. They also were frequency dependent, over the range 1 to 16 hertz, with the 16-hertz frequency producing peak pressure increases that were >90% of mean blood pressure, which was defined as a full erection. In a series of four control experiments, the mean frequency-ICP response patterns were reproducible and stable over at least five stimulation cycles, with little or no evidence of a diminution of the responses (fig. 2). A similar pattern was seen with internal pudendal artery blood flow and vascular resistance. Blood pressure and heart rate remained unchanged. The rises in ICP and penile blood flow produced a pronounced elongation of the penis and an increase in volume that was most pronounced in the glans and corpus spongiosum.

Four dogs were treated 5 minutes prior to stimulation of the pelvic nerve with the NO synthase inhibitor L-NOArg at doses of 100 μg./kg. to 3 mg./kg. This compound produced marked, dose-related rightward shifts in the frequency-response curves for ICP. After 3 mg./kg., L-NOArg completely blocked the response up to 4 hertz and reduced the 8 hertz response by more than 90% (fig. 3). The frequency-response curves for peak pudendal artery flow also were shifted to the right (data not shown). Measures of the in vivo potency of L-NOArg on ICP and blood flow parameters indicated that L-NOArg inhibited NO synthase, resulting in the blockade of electrically stimulated increases in ICP and blood flow. Also, the 'pseudo pA2' values, calculated by Schild analysis, had slopes close to 1, suggesting competitive inhibition of the enzyme (see table). In addition, L-NOArg produced a slight, dose-dependent increase in mean blood pressure (data not shown), possibly resulting from decreases in vascular endothelial NO production.

Sildenafil, 1 to 100 μg./kg., had no direct effect on basal ICP in any of the dogs but produced a dose-dependent potentiation of the increases in ICP produced by electrical stimulation. Pressure tracings from a typical experiment are shown in fig. 4, and the mean frequency-response curves for the control and the 1, 10, and 100 μg./kg. doses of sildenafil

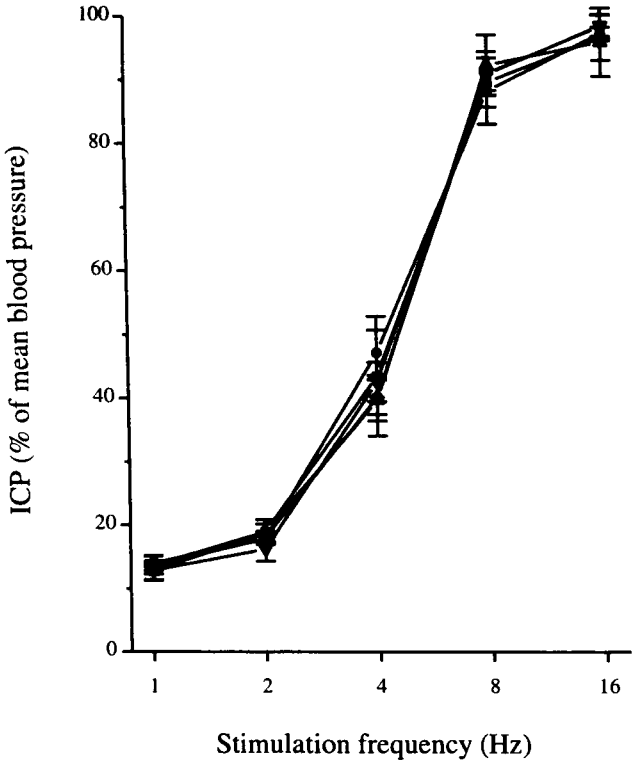

FIG. 2. Effect of five repeated stimulation cycles on intracavernosal pressure (ICP) induced by 1 minute of pelvic nerve stimulation at 10–15 volts, with 2 millisecond pulse width, at frequency of 1–16 hertz. Values represent mean ± standard error of 4 dogs.

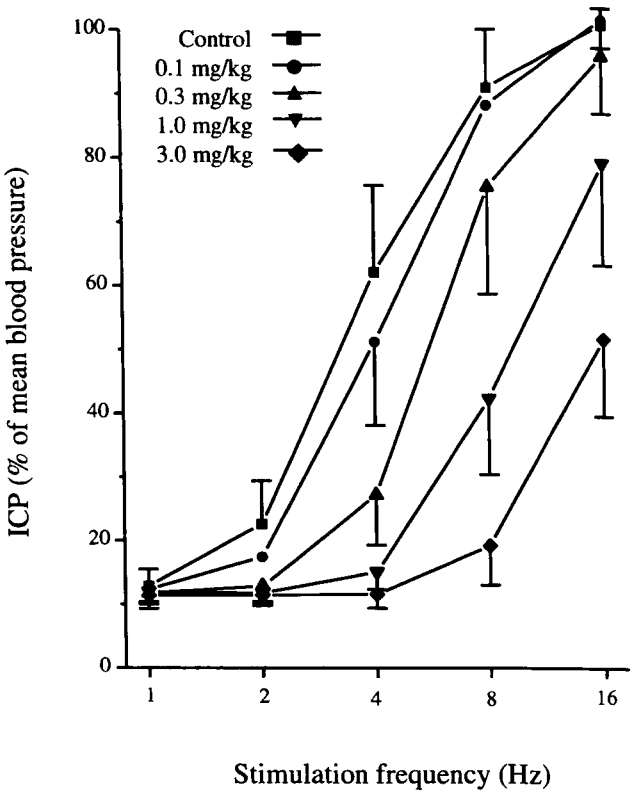

FIG. 3. Mean effect (± standard error) of various concentrations of L-NOArg on frequency-response curve for intracavernosal pressure (ICP) in anesthetized dog (n = 4).

are shown in fig. 5. The frequency-response curves for the 10 and 100 μg./kg. doses of sildenafil were significantly different from that of the control using analysis of variance ($p < 0.01$ and $p < 0.001$, respectively), indicating a marked potentiation. However, the pattern of the potentiation of ICP by sildenafil varied among dogs, with each dog having an optimum, most sensitive, stimulation frequency (that is, 2 to 8 hertz). Following sildenafil administration, the ICP at these frequencies was increased approximately 2–4-fold. When the mean effects of sildenafil on ICP at the optimum stimulation frequencies were calculated (fig. 6), ICP increases were significantly potentiated at sildenafil doses of 10, 30, and 100 μg./kg. By assuming that the maximum degree of potentiation, at the most sensitive frequency, occurs at the highest sildenafil dose used for each dog, an ED_{50} value (dose producing half maximum potentiation) could be estimated. For the five dogs, the mean ED_{50} value was 12.0 ± 2.0 μg./kg. Interestingly, sildenafil had no statistically significant effect on the rise in internal pudendal artery blood flow produced by stimulation at the optimal frequencies for ICP or any other frequency (data not shown).

Plasma concentrations of sildenafil were 13.7 ± 1.2 and 49.0 ± 2.6 ng./ml. 15 minutes following the 30 and 100 μg./kg. sildenafil doses, respectively. The other doses of sildenafil yielded plasma concentrations that were below the 4 ng./ml. detection limit of the assay. Finally, sildenafil produced no change in either blood pressure or heart rate.

Effect of NO synthase inhibitor L-NOArg on electrically stimulated intracavernosal pressure and penile blood flow values

Parameter	'Pseudo' pA_2 ± SE	Slope ± SE
Increase in intracavernosal pressure	5.53 ± 0.11	0.90 ± 0.07
Increase in pudendal artery blood flow	5.65 ± 0.13	0.98 ± 0.13

Affinities were determined by Schild analysis of dose ratios derived from shifts in the frequency-response curves to pelvic nerve stimulation (1–16 hertz). Values represent mean ± standard error (SE).

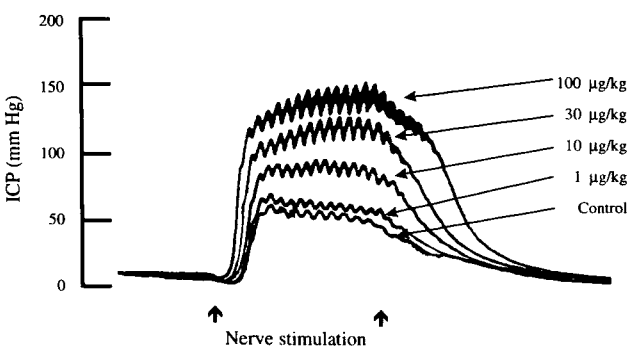

FIG. 4. Representative increase in intracavernosal pressure (ICP) produced by sildenafil at doses of 0 (control), 1, 10, 30, and 100 μg./kg. in dog stimulated at frequency of 8 hertz.

DISCUSSION

It has been well established that the corpus cavernosal smooth muscle relaxation required for erectile function is mainly, if not totally, dependent on the neuronal NO/cGMP system.[5] Many studies have shown the potentiating effects of NO donors and the inhibiting effects of NO synthase inhibitors and NO blockers on erectile mechanisms, both in vitro[15–17] and in vivo.[8–14] The importance of NO in the physiology of erectile function and on the effects of pelvic nerve stimulation is clearly supported by the results obtained with the NO synthase inhibitor L-NOArg in the present study.

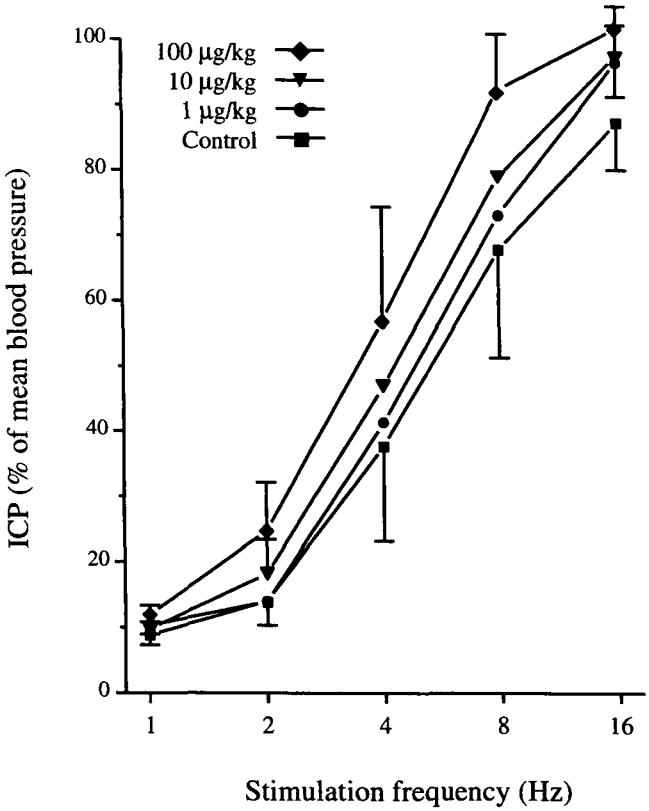

FIG. 5. Mean effect (± standard error) of sildenafil on frequency-response curve for intracavernosal pressure (ICP) in anesthetized dog (n = 5). Curves for 10 and 100 µg./kg. dose levels were significantly different from control curve, $p < 0.01$ and $p < 0.001$ respectively. (Curves for 3 and 30 µg./kg. doses have been omitted for clarity.).

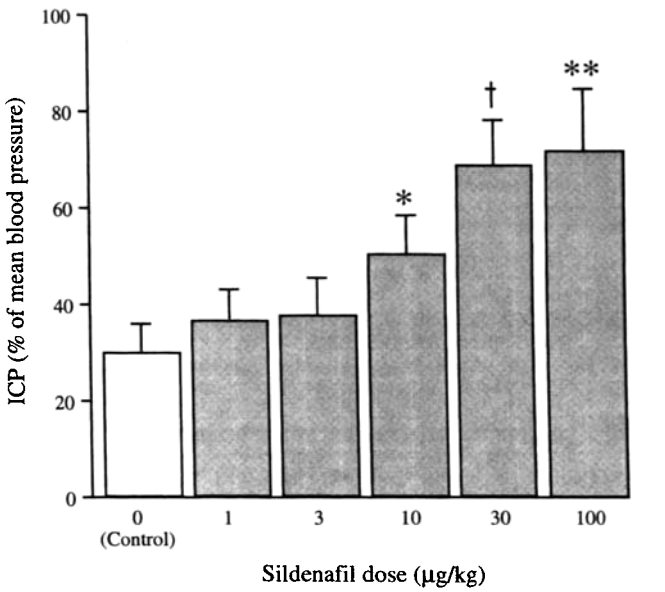

FIG. 6. Mean effect (± standard error) of sildenafil on intracavernosal pressure (ICP) increases induced by electrical stimulation at optimum frequency in anesthetized dogs (n = 5). * $p < 0.01$ versus control; † $p < 0.005$ versus control; ** $p < 0.001$ versus control using Student's *t* test.

Neuronally released NO exerts its effect by diffusing into the adjacent smooth muscle cells and activating soluble guanylate cyclase and increasing levels of intracellular cGMP, resulting in smooth muscle relaxation.[15,16] Thus sildenafil, a selective cGMP-specific PDE5 inhibitor, would be expected to amplify this mechanism by blocking cGMP metabolism and further elevating cGMP levels. The PDE5 inhibitor zaprinast, administered intracavernosally in the anesthetized dog, has been reported to potentiate the effects of nerve stimulation, giving support to this argument.[11]

There is some evidence in the literature that factors other than NO may be involved in penile erection, such as vasoactive intestinal polypeptide (VIP) acting through cAMP.[18] However, it has also been reported that blockade of adenylate cyclase did not reduce the response to pelvic nerve stimulation in the dog.[12] Thus, the role of VIP is unclear.

In the dog model of penile erection described here, sildenafil potentiated the increases in ICP induced by nerve stimulation without having any direct effect on ICP or potentiating penile blood flow parameters. These effects occurred at sildenafil doses that had no effect on blood pressure or heart rate. However, this potentiation did not occur uniformly across all frequencies; as a proportion of the control response it was most marked at 2–8 hertz, with the most sensitive frequency varying from dog to dog. However, the inter-dog variability seen in the present studies was not unexpected. Takahashi and associates, studying the effects of intracavernosal adenosine on penile erection in the dog, found a 22-fold variability in the dose required to produce a full response.[19] Although that study did not involve an NO system, it does show the potential inter-dog variability of cavernosal pressure responses.

ICP values increased at least 2-fold following treatment with sildenafil at doses of 30 to 100 µg./kg., which corresponded to peak plasma concentrations of 13.7 to 49.0 ng./ml. or 29 to 103 nM. In the dog, sildenafil has been found to be 86% protein bound in plasma, giving a free fraction of 14% (unpublished data). Thus, following treatment with 30 and 100 µg./kg. doses of sildenafil, the free plasma concentrations would be approximately 4 nM and 14 nM, respectively. These plasma concentrations correspond with in vitro data that indicate that sildenafil dose-dependently enhanced electrical field-stimulated relaxation of human corpus cavernosum at concentrations ranging from 1 nM to 1 µM.[20] In addition, the estimated ED_{50} value of 12.0 µg./kg. corresponds with a sildenafil plasma concentration of approximately 14 nM or 2 nM free drug, which is broadly consistent with the IC_{50} value of 4 nM for PDE5 inhibition by sildenafil.[6,20]

In recent studies sildenafil has been shown to potentiate the ICP rises induced by intracavernosal injection of the nitric oxide donor sodium nitroprusside (SNP) in the anesthetized dog. This enhancement occurred at a similar potency to that obtained with nerve stimulation, with a free drug plasma concentration at the ED_{50} value of 2.2 nM.[21] Sildenafil enhanced the NO-induced ICP rises in the absence of electrostimulation suggesting that it acts locally in the corpus cavernosum to produce its pro-erectile effects.

CONCLUSIONS

In an anesthetized dog model of erectile function, sildenafil potentiated the increases in ICP induced by pelvic nerve stimulation at dose levels resulting in sildenafil plasma concentrations consistent with its in vitro activity and PDE5 inhibition. The frequencies 2 to 8 hertz, where maximum sildenafil-induced potentiation occurred, were (importantly) also those most sensitive to inhibition by the NO synthase inhibitor L-NOArg. Thus, sildenafil augments the normal physiological NO/cGMP mechanism of penile erection, and has the potential to be a significant advance in ED treatment options.

REFERENCES

1. Jeffcoate, W. J.: The investigation of impotence. Br. J. Urol., **68:** 449, 1991.
2. NIH Consensus Development Panel on Impotence.: Impotence. Int. J. Impotence Res., **5:** 181, 1993.
3. Montorsi, F., Guazzoni, G., Rigatti, P. and Pozza, G.: Pharmacological management of erectile dysfunction. Drugs, **50:** 465, 1995.
4. Montague D. K., Barada J. H., Belker A. M., Levine L. A., Nadig P. W., Roehrborn C. G., Sharlip I. D. and Bennett A. H.: Clinical guidelines on erectile dysfunction: summary report on the treatment of organic erectile dysfunction. J. Urol., **156:** 2007, 1996.
5. Burnett A. L.: Role of nitric acid in the physiology of erection. Biol. Reprod., **52**: 485, 1995.
6. Boolell, M., Allen, M. J., Ballard, S. A., Gepi-Attee, S., Muirhead, G. J., Naylor, A. M., Osterloh, I. H. and Gingell, C.: Sildenafil: an orally active type 5 cyclic GMP-specific phosphodiesterase inhibitor for the treatment of penile erectile dysfunction. Int. J. Impotence Res., **8:** 47, 1996.
7. Boolell, M., Gepi-Attee, S., Gingell, J. C. and Allen, M. J.: Sildenafil, a novel effective oral therapy for male erectile dysfunction. Br. J. Urol., **78:** 257, 1996.
8. Azadzoi, K. M. and De Tejada, I. S.: Hypercholesterolemia impairs endothelium dependent relaxation of rabbit corpus cavernosum smooth muscle. J. Urol., **146:** 238, 1991.
9. Holmquist, F., Stief, C. G., Jonas, U. and Andersson, K-E.: Effects of the nitric oxide synthase inhibitor N^G-nitro-L-arginine on the erectile response to cavernous nerve stimulation in the rabbit. Acta Physiol. Scand., **143:** 299, 1991.
10. Meyer, M. F., Jahier, A., Krah, H., Staubesand, J., Becker, A. J., Kircher, M., Mayer, B., Jonus, U., Forssmann, W. G. and Stief, C. G.: Intracavernous application of SIN-I in rabbit and man: Functional and toxicological results. Ann. Urol., **27:** 179, 1993.
11. Trigo-Rocha, F., Aronson, W. J., Hohenfellner, M., Ignarro, L . J., Rajfer, J. and Lue, T. F.: Nitric oxide and cGMP: mediators of pelvic nerve-stimulated erection in dogs. Am. J. Physiol., **264:** H419, 1993.
12. Trigo-Rocha, F., Hsu, G. L., Donatucci, C. F. and Lue, T. F.: The role of cyclic adenosine monophosphate, cyclic guanosine monophosphate, endothelium and nonadrenergic, noncholinergic neurotransmission in canine penile erection. J. Urol., **149:** 872, 1993.
13. Wang, R., Higuera, T. R., Sikka, S. C., Minkes, R. K., Bellan, J. A., Kadowitz, P. J., Domer, F. R. and Hellstrom, W. J.: Penile erections induced by vasoactive intestinal peptide and sodium nitroprusside. Urol. Res., **21:** 75, 1993.
14. Trigo-Rocha, F., Hsu, G-L., Donatucci, C. F., Martinez-Pineiro, L., Lue, T. F. and Tanagho, E. A.: Intracellular mechanism of penile erection in monkeys. Neurourol. Urodyn., **13:** 71, 1994.
15. Ignarro, L. J., Bush, P. A., Buga, G. M., Wood, K. S., Fukuto, J. M. and Rajfer, J.: Nitric oxide and cyclic GMP formation upon field stimulation cause relaxation of corpus cavernosum smooth muscle. Biochem. Biophys. Res. Commun., **170:** 843, 1990.
16. Bush, P. A., Aronson, W. J. Buga, G. M., Rajfer, J. and Ignarro, L. J.: Nitric oxide is a potent relaxant of human and rabbit corpus cavernosum. J. Urol., **147:** 1650, 1992.
17. Knipsel, H. H., Goessl, C. and Beckman, R.: Nitric oxide mediates relaxation in rabbit and human corpus cavernosum smooth muscle. Urol. Res., **20:** 253, 1992.
18. Juenemann, K-P., Lue, T. F., Luo, J-A., Jadallah, S. A., Nunes, L. L. and Tanagho, E. A.: The role of vasoactive intestinal polypeptide as a neurotransmitter in canine penile erection: a combined in vivo and immunohistochemical study. J. Urol., **138:** 871, 1987.
19. Takahashi, Y., Ishii, N., Lue, T. F. and Tanagho, E. A.: Effects of adenosine on canine penile erection. J. Urol., **148:** 1323, 1992.
20. Ballard, S. A., Burslem, F. M. F., Gingell, C. J. C., Price, M. E., Tang, K., Turner, L. A. and Naylor, A. M.: In vitro profile of UK-92,480, an inhibitor of cyclic GMP-specific phosphodiesterase 5 for the treatment of male erectile dysfunction. J. Urol., **155:** 676A, 1996 [abstract].
21. Carter, A. J., Ballard, S. A. and Naylor, A. M.: Effects of sildenafil on intracavernosal pressure rises induced by injection of sodium nitroprusside into the corpus cavernosum in the anaesthetised dog. Presented at the second meeting of the European Society for Impotence Research, 1997.