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Chronic Sildenafil Improves Erectile Function and Endothelium-dependent Cavernosal Relaxations in Rats: Lack of Tachyphylaxis☆

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Abstract

Objectives: Sildenafil is a widely-prescribed effective on-demand treatment of erectile dysfunction (ED). Chronic treatment with sildenafil could help patients with ED.

Methods: The effects of an 8-week long treatment with sildenafil (60 mg/kg/d *sc*) in male Sprague Dawley rats were evaluated on electrically-elicited erectile responses *in vivo* before and after an acute injection of sildenafil (0.3 mg/kg *iv*). In addition, endothelium-dependent and -independent relaxations of strips of corpus cavernosum *in vitro* were examined. All experiments were performed 36 hours after the last injection of sildenafil.

Results: Endothelium-dependent relaxations of cavernosal strips to acetylcholine were enhanced after chronic treatment with sildenafil while relaxations to A23187 or sodium nitroprusside were unchanged. Frequency-dependent erectile responses elicited by cavernous nerve stimulation were significantly improved. Moreover, the erectile responses to acute sildenafil were greater in chronically-treated rats with sildenafil.

Conclusions: This is the first report providing experimental support for chronic dosing with sildenafil which could be of use for patients that are poor responders to on-demand treatment. Chronic sildenafil may regulate the transduction pathway leading to the activation of eNOS but has no effect on NO bioavailability or on the cGMP pathway, thereby eliminating a possible concern for tachyphylaxis.

Keywords: Erectile dysfunction; Sildenafil; PDE-5; Tachyphylaxis; Chronic administration; Corpus cavernosum; Endothelium

1. Introduction

Upon sexual stimulation, penile erection, occurring in response to the activation of pro-erectile autonomic pathways, is greatly dependent on adequate inflow of blood to the erectile tissue and requires coordinated arterial endothelium-dependent vasodilatation and sinusoidal endothelium-dependent cavernosal smooth muscle relaxation [1]. NO is the principal peripheral pro-erectile neurotransmitter, released both by parasympathetic-nitrergic autonomic nerves and the sinusoidal endothelium to produce cGMP and relax cavernosal smooth muscle, and resulting ultimately in increased intracavernosal pressure (ICP) [2]. Sildenafil inhibits selectively type 5 cGMP-phosphodiesterase (PDE-5), resulting in an enhanced availability of cGMP in cavernosal tissues, relaxation of smooth muscle and increased blood flow into cavernosal spaces. Sildenafil was the first orally active drug to

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treat patients with erectile dysfunction (ED) and has become the standard treatment for ED in more than 110 countries [3].

Although sildenafil has proven to be effective in treating ED, a report has suggested that its prolonged use may produce tachyphylaxis [4]. However, other clinical trials including more patients followed for longer periods of time have demonstrated only a small (less than 5%) patient dropout rate for lack of efficacy [5,6]. These low rates of withdrawal imply that men continued to be satisfied with sildenafil during long-term treatment. Furthermore, tachyphylaxis, in most pharmacological and human settings, does not occur with drugs that are used in an on-demand basis.

Nonetheless, recent data has suggested that sildenafil could have additional and prolonged beneficial effects on endothelial function in diabetic patients if taken on a daily basis [7]. Furthermore, the use of chronic sildenafil could also improve nocturnal erectile activity, thus maintaining the morphodynamic integrity of smooth muscle cells within the corpora cavernosa [8]. Finally, despite broad clinical success of sildenafil in the treatment of ED, certain patients with severe ED i.e. Erectile Function Domain Scores between 1 and 10 [9] remain poor responders. Our hypothesis is that chronic treatment, far from inducing tachyphylaxis, could help salvage non-responders to sildenafil therapy. Thus, whether chronic treatment with sildenafil leads to tachyphylaxis is important to ascertain since it may have widespread implications, not only for sildenafil, but also for the other PDE-5 inhibitors, vardenafil and tadalafil if this is a class effect.

The primary goal of this study was, therefore, to study the effects of chronic treatment with sildenafil on the erectile function in rats by (1) evaluating the effects of a chronic treatment with sildenafil on cavernosal tissue endothelial reactivity, and (2) studying their erectile responses elicited by electrical cavernous nerve stimulation under anaesthesia. Secondly, we investigated the sensitizing effect a chronic treatment with sildenafil on electrically-induced erectile responses before and after an acute administration of sildenafil in anaesthetized rats.

2. Methods

2.1. Animals

Male Sprague-Dawley rats (Charles River, France, 180–220 g, $n = 44$) were housed 7 days prior to the experiments with free access to food and water. All procedures were performed in accordance with the legislation on the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 1996) and Animal Care regulations in force in France as of 1988.

2.2. Drugs and solutions

Krebs bicarbonate solution of the following composition was used ((mmol l^{-1}): NaCl 118.0; KCl 4.6; $CaCl_2$ 2.5; KH_2PO_4 1.2; $MgSO_4$ 1.2; glucose 11.1; $NaHCO_3$ 4.15; HEPES, 20.85, pH 7.2–7.4). The following drugs were used: phenylephrine, acetylcholine, A23187, sodium nitroprusside (Sigma, St Louis, USA), sildenafil mesylate (Pfizer, Sandwich, UK).

2.3. Chronic treatment of rats

Each experimental group received 20 mg/kg of sildenafil mesylate (or saline solution) via subcutaneous injection repeated three times a day (60 mg/kg/d in total) during 8 weeks. Body weight was recorded every day. Blood samples were collected before treatment, during the treatment period (bi-monthly, 6–8 h after a subcutaneous injection) and at the time of the *in vivo* experiments for the determination of free plasma concentrations of sildenafil (UK-92,480) and its active metabolite (UK-103,320), as previously described [10]. All experiments were performed after a 36-h wash-out period.

2.4. Isometric tension studies in cavernosal strips

Rats were terminally anaesthetized with sodium pentobarbital (50 mg/kg *ip*). The penis was carefully harvested, transferred into ice-cold Krebs bicarbonate solution and dissected. Cavernosal strips were placed in 5-ml organ baths filled with Krebs maintained at 37 °C, bubbled with 95% O_2-5%CO_2 and connected to a force-displacement transducer. Tissues were equilibrated for 60 min. Concentration-response curves to acetylcholine (ACh, 10^{-9} mol/l to 10^{-4} mol/l), A23187, a calcium ionophore (10^{-9} mol/l to 3×10^{-5} mol/l) and sodium nitroprusside (SNP, 10^{-9} mol/l to 10^{-4} mol/l) were constructed on phenylephrine-induced pre-contracted tissues, as previously described [11].

2.5. Erectile responses to cavernosal nerve stimulation in anaesthetized rats

Erectile responses were elicited by electrical stimulation of the cavernous nerve in anaesthetized rats, as previously described [11]. Briefly, rats were anaesthetized with urethane (1.2 g/kg *ip*), tracheotomized and maintained at 37 °C. The carotid artery was catheterized to record arterial pressure and a 21-gauge needle was inserted into one of the corpus cavernosum of the penis to record ICP simultaneously via pressure transducers (Elcomatic 750, UK). The cavernous nerve was exposed at the lateral aspect of the prostate and mounted on a bipolar platinum electrode connected to an electrical stimulator (AMS 2100, Phymep, France). After stabilization, basal MAP was recorded. Electrical stimulations of the cavernous nerve (1 ms, 45 s, 6 V) at different frequencies (1, 2, 3, 4, 5 and 10 Hz) were performed in a randomized manner and repeated twice in order to establish frequency-response curves. Then, 4 minutes after an intravenous injection of sildenafil 0.3 mg/kg, electrical stimulations of the cavernous nerve at 1, 3 and 5 Hz were again performed before sacrifice of the rats with an overdose of urethane.

2.6. Calculations and statistical analysis

Values are expressed as mean ± S.E. mean of n experiments. Tissue contractile responses were expressed as the absolute change in maximal developed tension (in g) normalized per gram tissue weight and relaxations as the percentage of change in phenylephrine-induced tone. Concentrations inducing 50% of the maximal effect were expressed as pD_2 values and calculated using GraphPad Prism. Erectile responses elicited by electrical stimulation were expressed as the ratio of ΔICP (mmHg)/MAP (mmHg) × 100, with