Transient Rise of Serum Testosterone Level after Single Sildenafil Treatment of Adult Male Rats

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ABSTRACT

Introduction. Phosphodiesterase type 5 (PDE5) inhibitors have been established in therapy for a variety of physiological disorders including erectile dysfunction. Despite its popularity and wide usage in erectile dysfunction treatment, the short-term effect of PDE5 inhibition on Leydig cell functionality and testosterone dynamics is missing.

Aim. This study was designed to assess the acute in vivo effects of sildenafil citrate (Viagra) treatment on testosterone production.

Methods. Male adult rats were given sildenafil (1.25 mg/kg BW) per os, and testosterone production were analyzed 30, 60, 120, and 180 minutes after treatment. Additionally, in vitro effect of sildenafil extract on Leydig cell steroidogenesis was estimated.

Main Outcome Measures. The formation of testicular interstitial fluid (TIF), and testosterone, cyclic guanosine monophosphate (cGMP), cyclic adenosine monophosphate (cAMP) content was followed. Occurrence and phosphorylation of mature steroidogenic acute regulatory protein (StAR) and interaction with protein kinase G 1 (PRKG1) were assessed by immunoprecipitation and Western blot.

Results. Serum testosterone was increased 60 and 120 minutes after sildenafil treatment. In 60 minutes, TIF volume was doubled and stayed increased till the end of the experimental period. cGMP and testosterone content in TIF were increased 30 minutes after treatment, and cAMP decreased in 60 minutes. Further, sildenafil-induced stimulation of testosterone production was abolished by ex vivo addition of PRKG1 inhibitor but not by protein kinase A inhibitor. Sildenafil treatment increased the level of phosphorylated and total StAR protein. Moreover, co-immunoprecipitation of StAR and PRKG1 was increased following sildenafil treatment suggesting the active role of this kinase in initiation of testosterone synthesis. Additionally, sildenafil extract applied in vitro on primary Leydig cell culture increased cGMP accumulation and testosterone production in time- and dose-dependent manner without effect on cAMP level.

Conclusion. Acute sildenafil treatment enlarged TIF volume but also stimulated testosterone production which may be significant considering the positive testosterone effect in regulation of sexual activity. **Janjic MM, Stojkov NJ, Bjelic MM, Mihajlovic AI, Andric SA, and Kostic TS. Transient rise of serum testosterone level after single sildenafil treatment of adult male rats. J Sex Med 2012;9:2534–2543.**

Key Words. Testosterone; Sildenafil Citrate; cGMP; PDE5 Inhibitors; PRKG1; Leydig Cell

Introduction

Sexual function requires the complex interaction of multiple neurotransmitters and hormones, both centrally and peripherally, and sexual desire is considered the result of a complex balance between inhibitory and excitatory pathways in the brain. Testosterone has been claimed for so long as a pivotal hormone in regulating male sexual function, acting both at central and peripheral level [1]. In males, testicular Leydig cells are the major source of testosterone. The acute steroidogenic

response of Leydig cells is predominantly dictated by steroidogenic acute regulatory (StAR), a rapidly synthesized labile phosphoprotein whose expression, activation, and extinction is regulated by protein kinase A (PRKA), as well as a host of other signaling pathways [2]. StAR and other proteins of macromolecular signaling complex facilitate cholesterol transport across mitochondrial membranes delivering substrate to steroidogenic enzyme machinery that culminates with testosterone production. The cyclic guanosine monophosphate (cGMP) signaling is also operative in Leydig cells [3,4]. Stimulation of cGMP production either by nitric oxide (NO)-dependent soluble or membranous-bound guanylyl cyclases activates protein kinase G (PRKG) that could phosphorilates StAR protein in vitro and initiates steroidogenesis [5,6]. The Leydig cells express several cGMP hydrolyzing phosphodiesterases (PDEs) including PDE type 5 inhibitors (PDE5) [7,8] governing intracellular cGMP level [9]. Inhibition of Leydig cell PDE5 activity in vitro was accompanied by accumulation of cGMP and elevation of testosterone production [6]. Additionally, long-term in vivo sildenafil treatment changed PDEs expression pattern and stimulated testosterone production due to coordinative stimulatory action of cyclic adenosine monophosphate (cAMP) and cGMP [9].

Currently the first-line therapy of erectile dysfunction that successfully targets the cGMP-signaling pathway is oral usage of PDE5 inhibitors [10]. Because functional cGMP-signaling pathway is expressed in Leydig cells, it is of importance to establish their vulnerability to single treatment in a way that it is used in erectile dysfunction treatment.

This study was designed to evaluate the acute effects of most frequently used drug for erectile dysfunction treatment, sildenafil citrate on testosterone dynamics within testes in male rat. Results indicated testes and Leydig cells as targets for acute sildenafil action. The transient rise of testosterone circulatory level despite swollen testicular interstitial fluid (TIF) after single sildenafil usage indicated stimulated production which may be significant considering the positive testosterone effect in regulation of sexual activity.

Materials and Methods

Materials

PRKG inhibitor, KT5823, was obtained from Calbiochem (San Diego, CA, USA; http://www.calbiochem.com) while PRKA inhibitor, H89, was obtained from Sigma (St. Louis, MO, USA; http://www.sigmaaldrich.com). NO donor, DPTA (Dipropylenetriamine; 3, 3-(hydroxynitrosohydrazino)bis-1-propanami-ne) was from Alexis Biochemical (San Diego, CA, USA). The antisera for StAR protein was a generous gift from Professor Douglas Stocco [11], whereas purified antibody against phospho-StAR peptide was a gift from Steven R. King [12]. Purified rabbit polyclonal antibody against PRKG1 was obtained from Calbiochem (San Diego, CA, USA; http://www.calbiochem.com), while β-actin detection kit was from Oncogene Research Product (San Diego, CA, USA; http://www.emdbiosciences.com). Anti-rabbit secondary antibody linked to the horseradish peroxidase was obtained from Kirkegaard & Pery Labs (Gaithersburg, MD, USA; http://www.kpl.com). Sildenafil (Viagra) was from Pfizer (New York, NY, USA; http://www.Pfizer.com). All other reagents were of analytic grade.

Methods

Ethical Approval

All the experimental protocols were approved by the local Ethical Committee on Animal Care and Use at the University of Novi Sad operating under the rules of National Council for Animal Welfare and following statements of National Law for Animal Welfare (copyright March 2009). All our experiments were performed and conducted in accordance with the National Research Council publication *Guide for the Care and Use of Laboratory Animals* (copyright 1996, National Academy of Sciences, Washington, DC) and *NIH Guide for the Care and Use of Laboratory Animals* (NIH Publications no. 80 23, revised 1996, 7th edition). All the experiments were carried out in the Laboratory for Reproductive Endocrinology and Signaling, Department of Biology and Ecology, Faculty of Sciences at the University of Novi Sad.

Animals and Treatment

Adult (3 months old, 250–270 g body weight) male Wistar rats, bred and raised in the Animal Facility of the Faculty of Sciences (University of Novi Sad), were used for the experiments. The animals were raised in controlled environmental conditions (22 ± 2°C; 12-hour light/dark cycle, lights on at 7 AM) with food and water *ad libitum*. Animals were divided into five groups; each consists of five adult male rats. The first, control group, received pure distilled water by oral dosing and the other four, sildenafil-treated groups, received sildenafil