Superoxide Anion Production by NADPH Oxidase Plays a Major Role in Erectile Dysfunction in Middle-Aged Rats: Prevention by Antioxidant Therapy

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ABSTRACT

Introduction. Prevalence of erectile dysfunction (ED) increases progressively with aging, but the ED pathophysiology at its early stages is still poorly investigated.

Aim. This study aimed to evaluate the functional and molecular alterations of erectile function at middle age, focusing on the contribution of oxidative stress in erectile tissue for the ED.

Methods. Young (3.5-month) and middle-aged (10-month) male Wistar rats were used. Rat corpus cavernosum (RCC) was dissected free and mounted in 10-mL organ baths containing Krebs solution. Intracavernosal pressure (ICP) in anesthetized rats was evaluated.

Main Outcome Measures. Concentration–response curves to endothelium-dependent and endothelium-independent agents, as well as to electrical field stimulation (EFS), were obtained in RCC strips. Measurement of cyclic guanosine monophosphate (cGMP) and expressions of neuronal nitric oxide synthase (nNOS) and endothelial NOS (eNOS), $gp91^{phox}$ and superoxide dismutase-1 (SOD-1) expressions in RCC were evaluated.

Results. ICP was significantly reduced in middle-aged compared with young rats. RCC relaxations to acetylcholine (10^{-8} to 10^{-2} M), sodium nitroprusside (10^{-8} to 10^{-2} M), sildenafil (10^{-9} to 10^{-5} M), BAY 41-2272 (10^{-9} to 10^{-5} M), and EFS (4–32 Hz) were decreased in middle-aged group, which were nearly normalized by apocynin (NADPH oxidase inhibitor; 10^{-4} M) or SOD (75 U/mL). Prolonged treatment with apocynin (85 mg/rat/day, 4 weeks) also restored the impaired relaxations in middle-aged rats. Relaxations to 8-bromoguanosine 3′,5′-cyclic monophosphate sodium salt (8-Br-cGMP; 10^{-8} to 3×10^{-4} M) remained unchanged between groups. Basal and stimulated cGMP production were lower in middle-aged group, an effect fully restored by apocynin and SOD. Protein expression of nNOS and phosphorylated eNOS (p-eNOS) (Ser-1177) reduced, whereas gp^{91phox} mRNA expression increased in RCC from middle-aged rats.

Conclusions. ED in middle-aged rats is associated with decreased NO bioavailability in erectile tissue due to upregulation of NADPH oxidase subunit $gp91^{phox}$ and downregulation of nNOS/p-eNOS. Antioxidant therapies may be a good pharmacological approach to prevent ED at its early stages. **Silva FH, Mónica FZ, Báu FR, Brugnerotto AF, Priviero FBM, Toque HA, and Antunes E. Superoxide anion production by NADPH oxidase plays a major role in erectile dysfunction in middle-aged rats: Prevention by antioxidant therapy. J Sex Med 2013;10:960–971.**

Key Words. Corpus Cavernosum; Oxidative Stress; Apocynin; Antioxidant Therapy; Erectile Dysfunction; Superoxide Dismutase

Introduction

Aging is a complex process with multiple alterations in the physiological structure and functional responses of the organism [1]. The aging process is well-known to cause an unbalance between reactive-oxygen species (ROS) production and antioxidant capacity of the tissues, leading to cell damage [2,3]. ROS include free radicals such as O_2^- and hydroxyl radicals, as well as

non-radicals such as hydrogen peroxide. Several potential ROS-generating systems exist, including NADPH oxidase, xanthine oxidase, uncoupled nitric oxide synthase (NOS), and the mitochondrial respiratory chain. The NADPH oxidase is a multi-subunit flavoprotein complex consisting of the cytosolic subunits $p40^{phox}$, $p47^{phox}$, and $p67^{phox}$, and small GTPase Rac and membrane-bound subunits $gp91^{phox}$ and $p22^{phox}$ [2]. The subunit $gp91^{phox}$ acts as a crucial catalytic subunit for the O_2^- production.

Erectile dysfunction (ED) is characterized by a persistent inability to achieve and/or maintain an erection sufficient for satisfactory sexual performance. Epidemiologic studies show that aging is an independent predictor of ED, irrespective of comorbid disease states such as cardiovascular diseases [4]. The incidence rate for ED is 25–30 cases per thousand person years and increases with age [5], being 29% in middle-aged men (40- to 49-year-old men) [6]. Clinical studies have indicated that ED should be considered an early clinical manifestation of risk factors for cardiovascular events including acute myocardial infarction [7,8]. Therefore, understanding the mechanisms regulating ED might be important to prevent future complications and for the development of effective ED therapies. A number of experimental studies have assessed the age-related ED in different experimental conditions [3,9], but few have investigated the pathophysiological alterations of corpus cavernosum at the middle age [10,11]. Recently, upregulation of NADPH oxidase was shown to be highly associated with ED in hypercholesterolemic mice, hypertensive and diabetic rats [12–15]. However, no detailed study has explored the role of NADPH oxidase associated with the pathophysiological alterations in aging-related ED. Since O_2^- excess can influence the biological activity of NO by reducing the NO bioavailability [16], we hypothesized that increases in O_2^- production in corpus cavernosum of middle-aged rats contributes to ED at this stage. Therefore, in the present study we have undertaken functional and molecular studies to evaluate the importance of oxidative stress in the erectile tissue to middle-age ED.

Material and Methods

Animals

All experimental procedures were conducted in accordance with institutional guidelines, and they were approved by the Ethical Principles in Animal Research by the Brazilian College for Animal Experimentation (COBEA). Male Wistar rats (3.5- and 10-month-old, young and middle-aged, respectively) were provided by Central Animal House Services (CEMIB) of University of Campinas (São Paulo). Animals were housed in temperature-controlled facilities on a 12-hour light/dark cycle with ad libitum food and water access. The average body weight of young and middle-aged rats was 411 ± 5 and 525 ± 8 g, respectively.

Intracavernosal Pressure (ICP) Measurement

Rats were anesthetized with an intraperitoneal injection of urethane (1.2 g/kg). The left carotid artery was cannulated to permit continuous monitoring of mean arterial pressure (MAP). ICP was recorded by cannulating the corpus cavernosal approximately 3–5 mm above the base of the penis and connected to a pressure transducer. The major pelvic ganglion (MPG) was identified by dissecting the fibrous capsule posterior to the intersection of the lateral lobes of the prostate and the vas deferens with cotton tips. The MPG was electrically stimulated with two platinum electrodes connected to a Grass S48 stimulator (Astro-Med Industrial Park, West Warwick, RI, USA). Electrical field stimulation (EFS) was conducted at 6 V, 1-millisecond pulse width, and trains of stimuli lasting 45 seconds at varying frequencies, with intervals of 3 minutes between the stimulation trains. Changes in ICP were recorded using a PowerLab 400 data acquisition system (LabChart-software, version 7.0, AD Instruments, Colorado Springs, CO, USA).

Functional Studies in Cavernosal Strips and Concentration–Response Curves

Rats were anesthetized with isoflurane and exsanguinated. Strips of corpus cavernosum were mounted in a 10-ml organ system containing Krebs solution at 37°C continuously bubbled with a mixture of 95% O_2 and 5% CO_2 (pH 7.4) and vertically suspended between two metal hooks. One hook was connected to a force transducer and the other acted as a fixed attachment point. Tissues were allowed to equilibrate for 60 minutes under a resting tension of 5 mN. Isometric force was recorded using a PowerLab 400 data acquisition system (Software LabChart, version 7.0, AD Instrument, Milford, MA, USA).

Cumulative concentration–response curves were constructed for the muscarinic agonist acetylcholine (ACh, 10^{-8} to 10^{-2} M), the NO donor