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Original article

# Effects of chronic L-DOPA administration on neurogenic and endothelium-dependent relaxation responses in rabbit corpus cavernosum

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ABSTRACT

*Background:* Dopamine is a crucial central neurotransmitter that plays a fundamental role in the autonomic and somatic components of penile reflexes in animals and humans. Similar to the erectile responses of dopamine, systemic administration of L-DOPA induces yawning and penile erection in some species. The possible effects of L-DOPA on nitric oxide (NO)-dependent and -independent non-adrenergic non-cholinergic (NANC) relaxation responses mediated by electrical field stimulation (EFS) and endothelium-dependent relaxation were investigated in this study.

*Methods:* Thirty-two adult albino male rabbits, in two- and four-week-treatment groups, were divided into three subgroups: control group (saline-injected) ($n = 4$), 3 mg/kg/day (low dose) L-DOPA-injected groups ($n = 6$) and 12 mg/kg/day (high dose) L-DOPA-injected groups ($n = 6$). After the intraperitoneal injection treatments, the corpus cavernosum tissues were placed in organ bath chambers. The EFS-mediated responses, and the concentration-response curve to carbachol, sodium nitroprusside (SNP), sildenafil were assessed.

*Results:* The two-week treatment with high-dose L-DOPA decreased the NO-dependent NANC relaxation responses, while there was no change in the low-dose two- and four-week treatment groups. The NO-independent NANC relaxation responses in the two-week groups decreased, and the responses in the four-week groups were unchanged when compared to the controls. The relaxation responses to carbachol showed no differences among all groups except for the high-dose four-week L-DOPA group. The relaxation responses of SNP and sildenafil were increased in all of the treatment groups when compared to the controls.

*Conclusions:* The observed increases in SNP- and sildenafil-induced responses, along with the decreased EFS-mediated responses, suggest increased sensitivity in the NO-signalling pathway following L-DOPA administration.



## Introduction

Penile erection and detumescence are complex and multifactorial haemodynamic processes involving relaxation and contraction, consecutively, of the corpus cavernosum smooth muscle. These series of events are under the control of the autonomic nervous system [1]. At the level of the central nervous system (CNS), the hypothalamic and limbic systems are responsible for the psychological components of penile erection. Erectogenic signals are released from these central areas to facilitate the spinal cord pathways, which lead to erection of the penis *via* peripheral mechanisms [2].

Dopamine is a critical central neurotransmitter that plays a fundamental role in the autonomic and somatic components of penile reflexes in animals and humans [3,4]. However, this does not mean that dopamine is only a central neurotransmitter. The dopaminergic receptors have been shown in peripheral locations, and significant physiological effects of dopaminergic agents on

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renal, adrenal, and cardiovascular function, have been documented [5–9]. In addition, investigations have suggested that dopamine exerts its pro-erectile effects *via* both central and peripheral dopamine receptors [10–13]. Similar to the erectile responses of dopamine, the systemic administration of various dopamine agonists (apomorphine, lisuride and quinelorane) induces yawning and penile erection in some species [14–16]. Dopaminergic drug treatments, such as L-DOPA and bromocriptine, are also associated with hypersexuality, as a rarely reported side effect in Parkinson's patients [17].

Peripherally, erection is initiated as a spinal reflex by the spinal cord stimulating neuronal nitric oxide synthase (nNOS) activity from the non-adrenergic non-cholinergic (NANC) nerve terminals. Subsequently, nitric oxide (NO) production *via* nNOS activity causes increased blood flow to the cavernosal tissue [14,15,17]. Endothelial NOS (eNOS) is then activated by the continued shear stress on the endothelial lining of the cavernosal sinusoidal spaces and arteries. Endothelial-derived NO is produced *via* eNOS, which sustains the continuity of the erection [15–17]. The NO produced by nerve terminals in the erectile tissue diffuses to the neighbouring vascular and trabecular smooth muscle, where the NO-cyclic guanosine monophosphate (cGMP) pathway is triggered [18]. NO binds to the soluble guanylate cyclase in smooth muscle cells, stimulating the synthesis of cGMP [19]. The increased intracellular cGMP causes corporeal smooth muscle relaxation [19].

The impact mechanism of L-DOPA (dopamine precursor) administration on erectile function, time-dependently and/or dose-dependently, has not been completely elucidated. The aim of the present study was to establish if whether L-DOPA administration contributes to NO-dependent and NO-independent NANC relaxation responses mediated by electrical field stimulation (EFS). For this purpose, nitrergic-mediated relaxant responses were investigated.

## Materials and methods

### *Animals*

The animal experiments in this study were conducted in accordance with Helsinki Declaration and approved by Gazi University Animal Research Ethics Committee (GÜET-12.025).

Thirty-two New Zealand albino adult male rabbits (3- to 4-month-old) were divided into two- and four-week treatment groups. In turn these were divided into three subgroups: daily intraperitoneal (*ip*) saline-injected group (control) ($n = 4$), daily *ip* 3 mg/kg (low dose) of L-DOPA-injected group ($n = 6$), and daily *ip* 12 mg/kg (high dose) of L-DOPA-injected group ($n = 6$).

Animals were fed with standard laboratory chow and tap water *ad libitum*. Temperature-controlled (23.2 °C) condition, and 12:12 h reverse light–dark cycle were provided. After the treatment periods, the rabbits were sacrificed with an overdose of pentobarbital.

Penis was disconnected from the trunk and corpora cavernosa erectile tissue was dissected carefully by removing the connective tissue in Krebs–Henseleit liquid buffer composed of (mM): NaCl, 118; KCl, 4.7; $CaCl_2{\cdot}2H_2O$, 1.3; $MgCl_2{\cdot}6H_2O$, 0.5; $Na_2HPO_4{\cdot}2H_2O$, 0.9; $NaHCO_3$, 24.9; and glucose monohydrate, 11; pH = 7.4. Each corporeal body was cut longitudinally to obtain four equal strips (3 mm × 3 mm × 15 mm).

### *Experimental procedure of organ bath*

Each strip was mounted between two-wire hooks in an organ bath containing 20 mL of Krebs–Henseleit solution bubbled with 95% $O_2$ and 5% $CO_2$, maintained at 37 °C. Under 2 g isometric resting tension, the tissues were washed in 15-min intervals during 1 h for equilibration. All strips were contracted with 124 mM KCl Krebs solution (NaCl, 42.7; KCl, 124; $NaHCO_3$, 25; $CaCl_2$-$2H_2O$, 1.26; MgCl-$6H_2O$, 0.5; $NaH_2PO_4$-$2H_2O$, 0.9; glucose monohydrate, 11) and then washed four times in 15-min intervals for new equilibration.

### *Assessment of the EFS-mediated responses*

After stabilization period, the strips were preincubated for 20 min with guanethidine ($10^{-6}$ M), atropine ($10^{-6}$ M) and indomethacin ($10^{-5}$ M) to eliminate adrenergic, cholinergic and prostaglandin mediated responses, respectively. The strips were contracted with concentration of $10^{-5}$ M phenylephrine (PE)-induced submaximal contraction (70–80% of maximal contraction) (Fig. 1).

The EFS-induced responses were provided by isometric force displacement transducers (FDT 10-A, COMMAT İletişim Co., Ankara, Turkey) connected to an online computer *via* a 4-channel transducer data acquisition system (TDA-97 TRANSDUCER DATA ACQUISITION SYSTEM, COMMAT İletişim Co., Ankara, Turkey) and applied *via* two-platinum wire electrodes set vertically within the organ bath at opposite sides of the suspended tissue. The EFS parameters were selected as 70 V with 1 ms duration in 10-s trains at various frequencies (0.5, 1, 2, 4, 8, 16, 32 Hz) at 2-min intervals to obtain isometric relaxations of corporeal erectile tissue.

Relaxation responses through various frequencies (0.5–32 Hz) of transmural EFS were obtained after the PE-induced contraction had reached a plateau (Fig. 1). Following the EFS responses, N-nitro-L-arginine methyl ester (L-NAME, NO synthase inhibitor) ($3 \times 10^{-4}$ M) was put in organ bath to eliminate nitrergic mediated responses and, allowed for incubation during 30 min and then the same frequencies of EFS were repeated (Fig. 1).

The EFS-mediated relaxation responses were confirmed by ω-conotoxin GVIA (binding N-type voltage-dependent calcium channel). Therefore, ω-conotoxin GVIA ($3 \times 10^{-7}$ M) was added to organ bath for 20-min incubation. The EFS-mediated responses were repeated following its incubation.

### *Nitrergic system assessment*

The strips were preincubated for 20 min with antagonists so that guanethidine and indomethacin for assessment of carbachol; guanethidine, atropine, indomethacin and L-NAME for assessment of sodium nitroprusside (SNP); guanethidine, atropine and indomethacin for assessment of sildenafil were used. After antagonists incubation, PE-induced submaximal contraction of the strips were obtained. Then cumulative concentrations of carbachol ($10^{-8}$–$10^{-6}$ M), SNP ($10^{-8}$–$3 \times 10^{-6}$ M), and sildenafil ($10^{-6}$–$3 \times 10^{-4}$ M) responses were implemented separately.

### *Assessment of papaverine-induced responses*

Papaverine-induced responses were studied after all experiments, as papaverine has permanent effect on corpus cavernosum smooth muscle. Guanethidine, atropine, indomethacin and L-NAME were used as antagonists in order to assess papaverine-induced responses. PE-induced contraction responses of the strips preincubated antagonists were treated $3 \times 10^{-4}$ M papaverine.

### *Data analysis*

The EFS-mediated and compound-mediated results are expressed as a percent of the response to PE-induced contraction, as mean ± SEM. The shifts in carbachol, SNP and sildenafil concentration-response curves were evaluated by comparing $pEC_{50}$ values (the negative logarithm of the concentration for the