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# Melatonin Improves Erectile Function in Rats With Chronic Lower Body Ischemia

Norifumi Sawada,[1,2] Masanori Nomiya,[1,3] Mona Zarifpour,[1] Takahiko Mitsui,[2] Masayuki Takeda,[2] and Karl-Erik Andersson[1]

## ABSTRACT

**Introduction:** Arterial occlusive disease is the leading cause of erectile dysfunction (ED). Using an established rat model we wanted to characterize the changes caused by atherosclerosis-induced chronic ischemia on penile structures and erectile function.

**Aim:** To investigate the effect of melatonin on these parameters.

**Methods:** Adult male Sprague-Dawley rats were divided into control, arterial injury (AI) and AI with melatonin treatment groups. AI and AI-melatonin groups underwent endothelial injury of the iliac arteries and received a 2% cholesterol diet following AI surgery for 8 weeks. AI-melatonin group rats received melatonin (20 mg/kg/day) orally for 8 weeks after AI. The control group received a regular diet. After 8 weeks, erectile function was tested. Corpus cavernosum (CC) tissues were processed for pharmacological and immunohistochemical studies, histological examination, and Western blotting.

**Main Outcome Measures:** Apomorphine test was performed to evaluate erectile function. Organ bath study was performed to measure the CC-contraction induced by KCl and phenylephrine, and relaxation induced by electrical field stimulation (EFS) and sodium nitroprusside (SNP).

**Results:** The number of erectile responses was significantly lower in the AI group (2.5 ± 0.5/hour) than in the control (5.0 ± 0.7/hour) and in the melatonin-treated groups (5.0 ± 0.3/hour). The responses to phenylephrine were lower in the AI-groups than in the controls, but there were no differences between control and AI-melatonin groups. SNP-induced relaxation in the AI-melatonin group was higher than in the AI, but lower than in control group. The EFS-elicited relaxation responses in the AI group were significantly lower than in the control and AI-melatonin groups. Compared to controls, CC tissues from the AI group showed significantly higher collagen content, and lower protein expression of eNOS and nNOS, and increased expression of iNOS. These changes were reduced or prevented by melatonin treatment.

**Conclusion:** Treatment with melatonin reduced/prevented functional and morphological changes induced by chronic ischemia on penile structure and function.



**Key Words:** Arterial Occlusive Disease; High-Cholesterol Diet; Chronic Penile Ischemia; Oxidative Stress; Erectile Dysfunction; Melatonin

## INTRODUCTION

Penile erection is the end result of a complex neurovascular process in which nerves, endothelium of sinusoids and blood vessels, and smooth muscle cells in the target organ are involved.[1] Neurogenic nitric oxide (NO) is considered the most important factor for the relaxation of penile vessels and corpora cavernosa (CC) needed for an erectile response. It is established that the balance between contractant and relaxant factors controls the degree of tone of the penile vasculature and of the smooth muscle of the CC and determines the functional state of the penis.

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[1]Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA;

[2]Department of Urology, Interdisciplinary Graduate School of Medicine, University of Yamanashi, Chuo city, Yamanashi, Japan;

[3]Division of Bioengineering and LUTD Research, Nihon University School of Engineering, Koriyama, Fukushima, Japan



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Erectile dysfunction (ED) often occurs in association with aging, diabetes mellitus, hypercholesterolemia, and hypertension, causing reduced relaxation of the CC, especially through an impairment of the NO system.[1] Ischemia and consequent oxidative stress are believed to represent one of the causative factors.[2] A correlation between ED and hypercholesterolemia has been found in several studies,[3–5] and it is well known that vascular endothelial dysfunction occurs during the human aging process and is an independent risk factor for the development of atherosclerosis and hypertension. The abdominal aorta and its branches, especially the bifurcation of the iliac arteries, are involved earliest and most severely by atherosclerotic lesions.[6] Arterial occlusive disease (atherosclerosis) and concomitant chronic penile ischemia may produce ED, including an impaired relaxant effect of CC.[7] Despite intensive study in various animal models, the mechanisms behind the changes in erectile function caused by chronic penile ischemia are incompletely known.

Melatonin, the major secretory product of the pineal gland, has free radical scavenging and antioxidative properties against oxidative stress. Although melatonin has been reported to be able to increase all aspects of sexual activities in rats,[1] it also has been suggested that chronic administration of this hormone may lead to inhibition of male rat sexual activity.[8]

In previous studies in rats, we found that mechanically induced endothelial injury of the iliac arteries combined with a 2% cholesterol diet for 8 weeks produced arterial occlusive disease and lower body ischemia.[9] Using this model, we investigated whether chronic lower body ischemia-induced ED was associated with changes in contractility and morphology of the CC and if chronic treatment with melatonin could prevent functional and structural changes.

## METHODS

The experimental protocol, which complied with set guidelines for animal experiments, was reviewed and approved by the Animal Care and Use Committee, Wake Forest University.

### Experimental Design

Adult male Sprague-Dawley rats (440–500 g) were divided into arterial endothelial injury (AI), AI treated with melatonin (AI-melatonin), and control groups. The AI and AI-melatonin groups underwent mechanical endothelial injury of the iliac arteries and received a 2% cholesterol diet for 8 weeks (AI: n = 15; AI-melatonin: n = 10). To study the effect of melatonin (Sigma, St Louis, MO, USA), the AI-melatonin group was treated with melatonin at a daily dose of 20 mg/kg (gavage) for 8 weeks. A third group received a regular diet for 8 weeks and was used as an age-matched control group (n = 9).

An apomorphine test was performed without anesthesia in the 3 groups at 8 weeks after endothelial injury. After the apomorphine test, rats from each group were euthanized with $CO_2$ inhalation and thoracotomy, and the corporal tissues were harvested. The tunica albuginea was carefully opened from its proximal extremity of the CC toward the penile shaft, and the erectile tissue within the CC was microsurgically excised. One preparation (1 × 1 × 6 mm) was obtained from each CC. In addition, vessels from aorta to femoral arteries were removed and histologically examined.

### Mechanical AI of the Iliac Artery

The procedure for producing AI has been described previously.[9] Briefly, the animals were anesthetized with 3% isoflurane, and a 2Fr Fogarty arterial embolectomy catheter (E-060-2F) from Edwards Lifesciences LLC (Irvine, CA, USA) was passed through the femoral artery into the common iliac artery. The balloon was inflated with air and subsequently withdrawn from the common iliac artery to the femoral artery, a maneuver repeated 10 times on each side.

### Induction of Erectile Response by Apomorphine

Rats within the 3 groups (control: n = 6, AI: n = 7, AI-melatonin: n = 7) were injected with apomorphine (80 μg/kg), and after 5 minutes of stabilization, the number of erectile responses were observed for 1 hour. On the day of testing, rats were transferred from their home cage to a test chamber that the examiner could observe from below. Erectile responses were defined as an emerging gorging penis, usually followed by an upright posture, repeated pelvic thrusts, and genital grooming.

### Organ Bath Study of the CC

The rats were sacrificed by $CO_2$ asphyxia; the penises from 9 controls, 15 AI, and 10 AI-melatonin treated animals were dissected immediately, and 1 × 1 × 6 mm sections of the CC were prepared. The CCs were attached to tissue holders at one end and force transducers at the other in an organ bath system (Danish Myo Technology, Aarhus, Denmark) containing 15 mL of Krebs buffer aerated with 95% $O_2$/5% $CO_2$ at 37°C. CC strips were subjected to a resting tension of 100–150 mg and allowed to stabilize for at least 30 min. The signals were relayed to a physiography instrument (Powerlab ADI Instruments, Sydney, Australia) and measured. Chart 5 software (ADI Instruments) was used for real-time monitoring of tension. Krebs-Hanseleit (KH) solution was used for the organ bath at 37° and pH 7.4 and gassed continuously with 95% $O_2$/5% $CO_2$. The KH solution was changed approximately every 15 minutes and allowed to reach a stable condition. An equilibration time of 30 minutes was allowed before experimentation commenced. Cumulative contraction responses to phenylephrine (PE) and relaxant responses to the NO donor, sodium nitroprusside (SNP), were recorded. Relaxant effects were assessed in preparations contracted with 10 μmol/L PE.

Repetitive supramaximal electrical field stimulation (EFS) was achieved by placing parallel platinum electrodes on either side of the CC preparations. Stimulation duration was 10 seconds at 40-V amplitude, with 0.5-ms pulse width at frequencies of 2–32 Hz.