ORIGINAL ARTICLE

Effects of melatonin supplementary on the sciatic nerve conduction velocity in the ovariectomized-aged rat

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Submitted: June 26, 2007 *Accepted: August 20, 2007*

Key words: **melatonin; nerve conduction velocity; ovariectomy; estradiol; sciatic nerve**

Neuroendocrinol Lett 2007; **28**(5):666–670 **PMID:** 17984941 NEL280507A10

Abstract

OBJECTIVES: Melatonin is a potent antioxidant agent and an anti-aging hormone. Serum melatonin level declines during the menopause. Estradiol, a neuroprotective ovarian hormone, also decreases during the menopause. The purpose of this study is to evaluate the effect of melatonin supplementary on peripheral nerve function in the ovariectomized (OVX)-aged rats.

METHODS: Randomly selected OVX-aged Wistar rats received injections of melatonin (5 or 20 mg/kg) daily either two or six weeks. Nerve conduction velocities and distal latencies were determined from the propagation of action potential recorded by using an extracellular electrophysiological technique.

RESULTS: The mean distal latencies of melatonin-treated groups were shorter than that of the control group. Thus, the nerve conduction velocity was significantly greater in both two weeks and six weeks melatonin treated groups as compared to the controls ($p<0.001$).

CONCLUSION: Melatonin alleviates the electrophysiological properties of the sciatic nerve in OVX-aged rats. Thus, melatonin supplementary may have a potential clinical application for the treatment of postmenopausal peripheral nerve degeneration.

INTRODUCTION

Estradiol deprivation has been implicated as a risk factor in neurological disorders, and estradiol-mediated neuroprotection has been described in several *in vitro* model systems (Behl, 2002; Bhavnani, 2003; Cholerton *et al*. 2002). Estradiol can interact with neuroprotective intracellular signaling pathways and is itself a neuroprotective antioxidant. Estradiol serves as a free-radical scavenger in preventing nerve cell death induced by various oxidative insults (Bhavnani, 2003; Garcia-Segura *et al*. 2001).

Melatonin is the main pineal hormone that is commonly produced and secreted at night. Melatonin protects cells from oxidative stress which was represented by scavenging free radicals (Yon *et al*. 2006; Tan *et al*. 2002) and regulating the activity and expression of antioxidant enzymes (Swiderska-Kolacz *et al*. 2006; Antolin *et al*. 1996; Urata *et al*. 1999). Melatonin has an anticytotoxic and anticarcinogen activity (Anisimov *et al*. 2006, Di Bella and Gualano 2006). Furthermore, melatonin has a geroprotective effect and may exert for the prevention of premature aging (Anisimov *et al*. 2006).

Pappolla *et al.* (2002) demonstrated that the neuroprotective action of melatonin was likely related to the antioxidant properties of melatonin. Feng and Zhang (2005) reported that melatonin administration in OVX rats led to reduced oxidative stress and consequently reduced neuronal apoptosis.

OVX female rat model has been widely used to mimic postmenopausal pathophysiological changes in women (Sato *et al.* 2003). Therefore, we hypothesize that oxidative stress might be involved in postmenopausal electrophysiological changes. Melatonin as an endogenous antioxidant might somewhat protect neuropathic injury. In this paper, we used the OVX rat model to explore roles of melatonin on postmenopausal peripheral nerve degeneration.

MATERIAL AND METHODS

Melatonin was first dissolved in absolute ethanol to prepare stock solution. Then, final concentration of 5 and 20 mg/ml melatonin solutions were daily prepared by diluting stock solution with 0.9% saline. The Krebs solution containing 124 mM NaCl, 5 mM KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 2 mM $CaCl_2$, 26 mM $NaHCO_3$, and 10 mM glucose was used in the experiment. Deionized water was used to prepare the solutions. Krebs solution was bubbled with 95% O_2 + 5% CO_2. All chemicals were purchased from Sigma Chemical Co (St. Louis, MO, USA) and used as received.

Four months old adult female Wistar Albino rats (200±25 g; n=64) were obtained from the Experimental Animal Center, Adnan Menderes University, Aydin, Turkey. The protocol for the experiment was approved by the Adnan Menderes University's Animal Experimentation Ethics Committee. Adult female Wistar rats were kept in conventional room with controlled light (12:12, dark: light), temperature (22±1 °C), relative humidity (40–50%) and ventilation (15 air changes per hour). They had free access to standard laboratory feed and water *ad libitum*. They were allowed to adapt to their environment for 1 week prior to the experiments

All animals were anesthetized intraperitoneally with 50 mg/kg ketamine hydrochloride and 5 mg/kg xylazine. Then, bilateral ovariectomies were performed through flank incisions as previously described (Feng and Zhang, 2005).

Twelve months after the ovariectomy, animals were randomly divided into two main groups depending on treatment interval (either two weeks or six weeks treated groups). Each main group (n=24) was divided into three subgroups such as 5 mg/kg intraperitoneal (i.p.) melatonin injected group , 20 mg/kg i.p. melatonin injected group, and i.p. 5% ethanol saline injected group (control group). Also, only i.p. saline injected group (n=8) was added as another control group. By including two different control groups, the effect of ethanol-saline solution and i.p. injection were eliminated in this experimental setup. Body weights were monitored before and at the end of the treatment.

The sciatic nerves were dissected from rats under ether anesthesia before animals were sacrificed by decapitation. The sciatic nerves were placed carefully in Petri dishes, containing Krebs solution which was flushed with 95% O_2 + 5% CO_2. The nerves were freed of fat and connective tissue before placed on a 15 parallel stainless steel Ag/AgCl electrodes (5 mm interelectrode distance) in a plexiglass nerve chamber. This part of experiment was carried out at 37 °C. The width of the chamber was 5 cm and its length was 15 cm. Nerve conduction velocities were measured using the MP100 data acquisition and analysis system (BIOPAC Systems, Inc., Santa Barbara, CA). During the measurements, supramaximal stimulus (Single square pulse, 7 V, 1 ms duration) generated by a MP 100 stimulator was used to stimulate the nerves.

All data are expressed as the mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey test was used for multiple comparison. For a single comparison, the significances of differences between means were determined by paired t test. Values of $p<0.05$ were considered statistically significant in all evaluations.

RESULTS

There were significant differences between control and melatonin treated animals regarding distal latencies and conduction velocities ($p<0.001$). Mean distal latencies of experimental groups were shorter than that of the control group (Figure 1). There were no statistically significant differences among distal latencies of melatonin treated groups ($p>0.05$). Also, no statistically significant difference was found between the latencies of ethanol administrated groups ($p>0.05$).

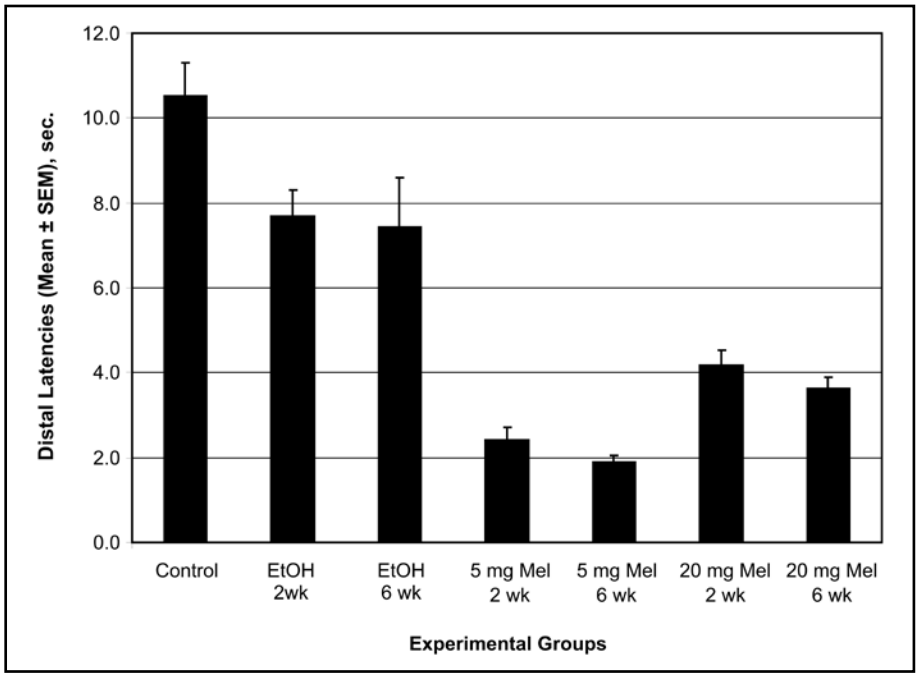

Figure 1. Distal latencies (Mean±SEM) of all experimental groups

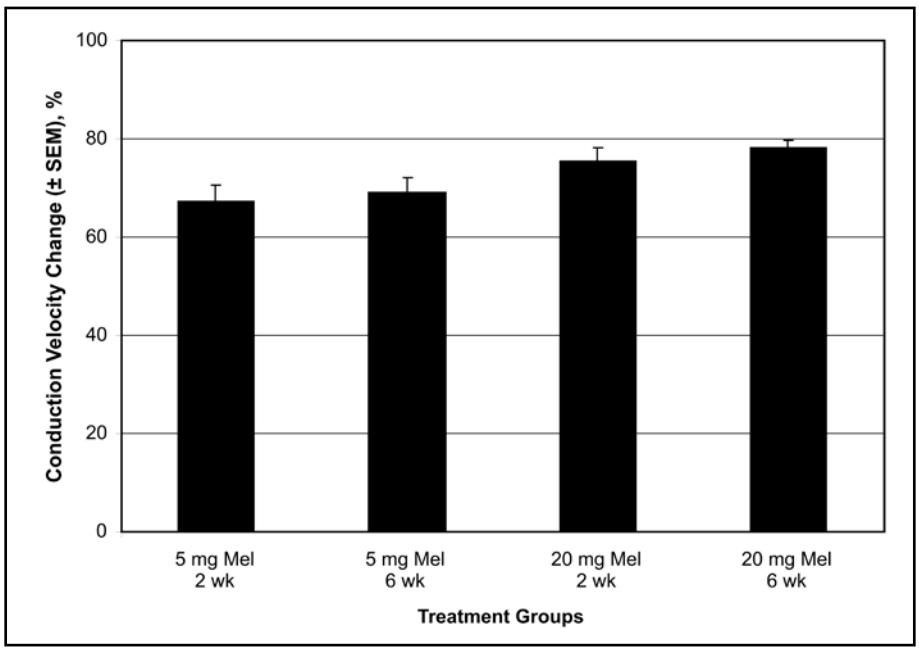

Figure 2. Conduction velocity changes in melatonin-treated groups compared to control group ($p>0.05$ among the melatonin treated groups)

Peripheral nerve conduction velocities in the OVX-aged rats increased approximately 68% and 77% in two- and six-weeks melatonin-treated groups, respectively (Figure 2). Increases were statistically significant in all melatonin-treated groups compared to the controls (control vs 5 mg Mel 2 wk, $p<0.05$; control vs 5 mg Mel 6 wk, $p<0.001$; control vs 20 mg Mel 2 wk, $p<0.001$; control vs. 20 mg Mel 6 wk, $p<0.001$). However, there was not any statistically significant change among the melatonin-treated groups ($p>0.05$).

Mean body weights of OVX-aged rats were decreased after melatonin treatment (Table 1). These differences were statistically significant ($p<0.05$). However, there were not statistically significant changes among the control groups (5% ethanol saline and saline injected groups, $p>0.05$).

DISCUSSION

Various pathological processes such as generalized or localized neuropathies, nerve injuries, pinched nerves, etc., were result in changes in latencies, motor and/or sensory amplitudes, or slowing of the conduction velocities to differing degrees. Therefore, nerve conduction

velocity (NCV), the speed of conduction of an electrical impulse through a nerve, is used to evaluate the damage and destruction of nerves and it may helpful to diagnosis certain diseases of the nerves.

Previous reports have shown that estrodiol has a wide range of actions in the nervous system, including neuroprotection and potentiation of nerve regeneration (Wise *et al*. 2001). However therapeutic applications of estrogen are severely limited because of its adverse side effects in reproductive organs. Islamov *et al.* reported that a positive role of estrogen on regeneration of peripheral nerves and in their study, systemic delivery of the estrogen significantly enhanced regeneration of the sciatic nerve in OVX female mice (Islamov *et al*. 2002). Also serum melatonin concentrations were sensitive to estrogen administration (Kerdelhue *et al*. 2006). Therefore the ovariectomized rat model was used to restrict the effect of estrogen during the melatonin treatment. Comelekoglu *et al.* (2005) reported that there were no statistically significant differences in the amplitude and area of compound action potential between OVX and control group. But, distal latency was significantly increased and subsequently the conduction velocity was reduced in OVX group. The similar results were also determined in our study. The conduction velocities were decreased in OVX rats compared to that of non-OVX rats (Data is not shown).

Ladizesky *et al*. (2003) suggested that ovariectomy augmented body weight, whereas melatonin treatment reduced it. Our present results support such an action of melatonin on body weight.

Melatonin enhances the in vitro and in vivo repair of severed rat sciatic nerve (Stavisky *et al*. 2005). Melatonin also has neuroprotective effect on experimental brain injury (Ates *et al*. 2006). Even though there are not traumatic injuries in sciatic nerve in OVX-aged rats, the sciatic nerves are affected by estradiol depletion in OVX-rats. Our data suggested that melatonin administration enhances nerve conduction velocity in the OVX-aged rat sciatic nerve. In contrast, there were no statistically significant differences in the conduction velocity between 5 mg/kg ip melatonin treatment for two weeks and 20 mg/kg ip melatonin treatment for six weeks ($p>0.05$). Feng and Zhang (2005) reported that the antioxidant effect of 5 mg/kg melatonin treatment was almost as strong as those at higher dose (20 mg/kg) in the OVX rat. Therefore, our results provide possible evidence that intermediate-term; low-dosage (5 mg/kg) melatonin administration could give beneficial effects for postmenopausal women.

Feng *et al.* (2004) reported that long-term supplementary of melatonin might alleviate the postmenopausal CNS learning and memory deficit in adult ovariectomized rats. However, the exact impacts of the melatonin supplementary on peripheral nerve functions in the OVX-aged rats remain unclear until to this study.

In conclusion, we demonstrate that melatonin supplementary alleviated the postmenopausal peripheral nerve degeneration. Thus it provides some evidence to justify the use of melatonin in clinical trials to treat postmenopausal women.

Table 1. Mean body weights of the experimental groups of OVX-aged rats.

Groups	Mean Body Weight ± SEM, [g]		p-value
	Before treatment	After Treatment	
5 mg Mel 2 wk	324.5±9.3	299.4±8.0	0.018
5 mg Mel 6 wk	356.2±13.5	315.5±11.5	0.017
20 mg Mel 2 wk	345.6±19.2	318.3±18.6	0.045
20 mg Mel 6 wk	323.8±17.6	274.5±13.9	0.027
EtOH 2 wk	317.5±13.1	301.6±9.7	0.115
EtOH 6 wk	305.8±12.2	290.3±4.8	0.185
Control	299.4±6.5	289.3±9	0.505

* Melatonin treatments cause statistically significant differences ($p<0.05$) on the mean body weight of experimental groups.

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