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Research report

Melatonin protects against neuronal damage induced by 3-nitropropionic acid in rat striatum

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Abstract

In this study, the protective effects of melatonin were evaluated against 3-nitropropionic acid (3-NP)-induced striatal neuronal damage in rats. Lesions were induced in the right striatum of Sprague–Dawley rats by stereotaxic injection with 3-NP and melatonin was intraperitoneally administered both 30 min before and 60 min after 3-NP injection. And rats continuously received melatonin daily for 3 days. As indicators of oxidative damage, lipid peroxidation and protein oxidation in the lesioned striatum were measured at 1 day after 3-NP injection. Levels of malondialdehyde (MDA) and protein carbonyl were significantly increased by 3-NP injection, but reduced in the melatonin-treated rats. Four days post-lesion, large lesions and extensive neuronal damage were produced in the 3-NP-injected striata, as revealed by 2,3,5-triphenyltetrazolium chloride (TTC) staining. In addition, marked ipsilateral rotational behavior following apomorphine challenge and a decrease of dopamine content in the lesioned striatum were observed in the 3-NP-injected rats. However, melatonin treatment significantly attenuated the 3-NP-induced neuronal damage, reduced the degree of asymmetric rotational behavior, and restored the dopamine level in the lesioned striatum. The present results indicate that melatonin effectively protects against the neuronal damage caused by 3-NP in vivo and that the neuroprotective effects of melatonin may be related to antioxidant action.

Theme: Motor systems and sensorimotor integration
Topic: Basal ganglia (mechanisms and models)

Keywords: 3-Nitropropionic acid (3-NP); Neuronal damage; Oxidative stress; Antioxidant; Melatonin

1. Introduction

Huntington's disease (HD) is an inherited, autosomal dominant, neurodegenerative disorder that leads to both motor and cognitive dysfunctions and is characterized by a progressive neurodegeneration of striatal neurons [32,37]. 3-Nitropropionic acid (3-NP) is a naturally occurring fungal toxin that is an irreversible inhibitor of succinate dehydrogenase (SDH) in the tricarboxylic acid cycle and electron transport chain complex II. This compound, when administered systemically, generates striatum-selective lesions and produces delayed dystonia and abnormal choreiform movements in rats and humans [25]. Experiments using 3-NP have therefore been conducted as an animal model of HD [3,8]. However, the neurotoxic mechanism of 3-NP is not well understood. There is a lot of evidence that the administration of 3-NP can produce selective striatal lesions that occur by secondary excitotoxic mechanisms [3,38], i.e., indirect activation of glutamate receptors [39]. Additionally, it has been reported

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that oxidative stress plays a substantial role in 3-NP-induced neuronal damage [20,34]. Systemic administration of 3-NP to rats depleted glutathione pools and increased the production of hydroxyl radicals and nitrotyrosine, as well as oxidized proteins, in the lesioned striatum [17]. Endogenous and exogenous antioxidants such as *N*-acetylcysteine, coenzyme Q10, and free radical spin trap 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) effectively protected against 3-NP-induced neuronal damage in rats [20]. These findings suggest that oxidative stress may be an important mediator of the neuronal damage produced by energy impairment or excitotoxicity and that inhibition of free radical processes can promote neuronal survival from 3-NP-induced neuronal damage.

Melatonin (*N*-acetyl-5-methoxytryptamine), a neurohormone that is synthesized in the pineal gland, is known to play roles in many physiological processes, such as photoperiod information, reproduction, immune response, and aging process [30]. Recently, melatonin has been found to possess free radical scavenging and antioxidant properties [31]. This antioxidant action of melatonin holds much interest in terms of its neuroprotective role in neurodegenerative disorders [31,32]. In the present study, we investigated whether melatonin can protect against the neuronal damage induced by 3-NP injection in vivo. With a focus on oxidative neuronal damage, we also examined the protective action of melatonin on the 3-NP toxicity.

2. Materials and methods

2.1. Rats and drug treatment

Nine-week-old male Sprague–Dawley rats (270–300 g) were maintained in a 12-h reversed light–dark cycle with free access to water and food. All rats were treated in accordance with the NIH Health Guidelines and the Korean Academy of Medicine Sciences for rat care and use. 3-NP was dissolved in saline and the pH was adjusted to 7.4 using 1 N NaOH. The rats were anesthetized with equithesin and 100 nmol 3-NP was stereotaxically injected into the right striatum at the following coordinates: AP +0.7 mm, ML +2.6 mm, DV −4.5 mm from the bregma and dura mater [10]. All injections were made in volumes of 4 μl each using a 26-gauge Hamilton syringe at a rate of 1 μl/min and the needle was left in place for 3 min before being slowly withdrawn [1]. The sham-lesioned rats were injected with 4 μl of saline, adjusted to pH 7.4, instead of 3-NP. Melatonin was dissolved in 5% ethanol/phosphate-buffered saline and 10 mg/kg melatonin was intraperitoneally administered both 30 min before and 60 min after 3-NP injection. The rats continuously received melatonin daily for 3 days after 3-NP injection. The same volume of vehicle was administered to both the sham-lesioned and 3-NP-injected rats.

2.2. Measurement of lipid peroxidation

One day after 3-NP injection, the rats were sacrificed by decapitation and the brains were rapidly removed, striatal tissue was immediately prepared and weighed, homogenated with 1 ml of 20 mM sodium phosphate buffer (pH 6.5) containing leupeptin (0.5 μg/ml), pepstatin (0.7 μg/ml), aprotinin (0.5 μg/ml), phenylmethylsulfonyl fluoride (40 μg/ml), and 1 mM EDTA. For measurement of thiobarbituric acid (TBA) reactive malondialdehyde (MDA) as a parameter of lipid peroxidation, 300 μl of homogenated tissue was mixed with a cocktail solution containing 8.1% of SDS, 20% acetic acid, and 0.67% TBA, shaken vigorously, boiled for 1 h, cooled with tap water, and centrifuged at 1500 × *g* for 10 min at room temperature. Supernatants were used to calculate the level of MDA by measuring absorbance at the 532-nm wavelength [4]. Concentrations are expressed as nmol MDA/mg protein.

2.3. Measurement of protein carbonylation

One day after 3-NP injection, the oxidation of proteins was measured in terms of the content of protein carbonyl by using the 2,4-dinitrophenylhydrazine (DNPH) spectrophotometric assay method [24]. The remaining homogenate fraction was divided into two equal aliquots. Both aliquots were precipitated with final 10% (wt/vol) trichloroacetic acid. One sample was treated with 2 N HCl, and the other with an equal volume of 0.2% (wt/vol) DNPH in 2 N HCl. Both samples were incubated at room temperature for 15–30 min in microcentrifuge tubes and mixed with vortex at 5-min intervals. The samples were then reprecipitated with 10% trichloroacetic acid at 18,000 × *g* at 4 °C. The precipitates were washed three times with ethanol/ethyl acetate (1:1, vol/vol). The final pellet was dried in a vacuum centrifuge and dissolved in 6 M guanidine HCl with 20 mM sodium phosphate buffer, at pH 6.5. Insoluble debris was removed by centrifugation for 3 min at 18,000 × *g* at 4 °C. The spectral difference between the DNPH-treated sample and the HCl control was determined, and the results are expressed as nmol of DNPH incorporated per mg protein based on the absorption of 21.0/mM/cm at 375 nm for aliphatic hydrazones. The protein concentration of the homogenate was determined by using the commercial assay from Pierce (Rockford, IL, USA). Bovine serum albumin was used as the standard.

2.4. Apomorphine-induced rotation test

To assess the motor asymmetry induced by unilateral injection of 3-NP, apomorphine (0.5 mg/kg) was subcutaneously injected 4 days post-lesion. The turning behavior was monitored for a duration of 60 min using automated rotometer bowls [36], and the motor asymmetry score was calculated as the number of net ipsilateral turns (turns