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Research report

Melatonin protects against behavioural dysfunctions and dendritic spine damage in 3-nitropropionic acid-induced rat model of Huntington's disease

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HIGHLIGHTS

- Melatonin protects against 3-NP-induced weight loss, behavioural dysfunctions in rat.
- Melatonin failed to correct memory loss, hanging ability.
- 3-NP-induced striatal glutamate increase, but not DA was ameliorated by melatonin.
- Melatonin attenuated dendritic spine loss in cortex and striatum.
- 3-NP-induced cerebellar granular cell arborization was protected.

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ABSTRACT

Huntington's disease (HD), an autosomal dominant neurodegenerative movement disorder in which striatal and cortical neurons are mostly affected, has no effective cure existing. A fungal neurotoxin and a potent inhibitor of mitochondrial electron transport chain complex II inhibitor, 3-nitropropionic acid (3-NP) is known to cause HD pathology, including lesions in the striatum and the cortex, and several behavioural syndromes in experimental animals. In the present study we examined the effect of melatonin on motor activities, neuronal morphology as revealed by Nissl and rapid Golgi staining, as well as GABA, glutamate and biogenic amine neurotransmitter levels in 3-NP-induced HD in rats. We found that melatonin (10, 20 mg/kg, i.p.) administered 1 h prior to 3-NP dose (20 mg/kg; daily for 4 days) restored motor coordination ability as shown in gait, beam balancing, swim ability and performance on rotarod. However it failed to reduce 3-NP-induced striatal lesion core area, neuronal damage and the elevated levels of striatal dopamine. Melatonin administration partially restored 3-NP-induced loss of dendritic spines in the striatum and the cortex, and the reduction in cerebellar granule cell, but not hippocampal CA1 neuronal arborization. These findings collectively suggest that melatonin offers beneficial effects in correction of learning related fine motor adjustments, but not in behaviours unrelated to learning, by the restoration of striatal and cortical spines, and cerebellar granule cell arborization.

1. Introduction

Huntington's disease (HD) is a neurodegenerative motor neuronal disease affecting the basal ganglia and associated structures. It is characterized by motor incoordination in the form of involuntary, rapid, jerky movements called chorea, and cognitive decline in advanced stages. The basic feature of this disease is the death of striatal medium spiny neurons, generally attributed to the presence of a mutated form of the ubiquitously expressed Huntingtin (Htt) protein. The mutation causes an increase in CAG repeats in the 5′ end of the huntingtin gene and the effect is inherited in an autosomal dominant manner [1]. Although several studies have attempted to find the underlying cellular and molecular reason for a site-specific lesion in the brains of HD patients or experimental animals, the outcome is still inconclusive. Some results indicate that striatal specific proteins like 'Rhes' are involved in this region specificity [2], whereas others imply that a region specific defect in the processing of the mutated Htt itself is crucial [3]. Standing out is the classical theory of dopamine (DA) neurotoxicity, which is based on the premise that the DA rich striatum contains high levels of its oxidized and hydroxylated metabolites, that inhibit mitochondrial

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respiratory chain culminating in oxidative injury to the neurons [4–7]. In fact, studies on human postmortem brain samples showed that complexes I–III and complex-II of the mitochondrial electron transport chain (ETC) complexes are affected in HD [8,9]. A recent review summarized mitochondrial functions and their biogenesis in relation to HD pathology, and treatment [10]. Direct association of the mutated Htt (mHtt) aggregates with mitochondria was demonstrated earlier, which may lead to membrane depolarization, affecting the overall functioning of this organelle [11–13].

As in many other neurodegenerative disorders, the involvement of mitochondria is said to be crucial in developing HD pathology. Blocking mitochondrial ETC complex II (succinate dehydrogenase) activity by 3-nitropropionic acid (3-NP) is shown to replicate HD-like symptoms in both primates and rodents leading to an extensive use of this toxin to develop a rat model of HD [14–17]. 3-NP-induced HD phenotypes in this animal model are shown to be better in terms of severity of symptoms at an advanced stage of the disease, and juvenile onset HD [18]. In short, the generalized features of this model are: striatal lesion formation, loss in mitochondrial ETC complex activity, imbalance in striatal neurotransmitter levels and increased oxidative stress, which are remarkably reminiscent of human HD [14,16–19]. Along with a loss in body weight, the animals' motor-behavioural aspects of gait, grip, ability to balance over a narrow beam, foraging or exploratory behaviours, cognition, anxiety or depression and swimming ability are mostly affected [17,18,20–26].

A neurohormone, melatonin is known as a potent free radical scavenger [27] and a blocker of the mitochondrial permeability transition pore [28,29]. There are a number of reports which suggest melatonin's neuroprotective effects in 3-NP-induced neurotoxicity in neuroblastoma cell lines [30] and in animal models of HD [19,21,31,32]. These studies generally point towards melatonin's ability to protect 3-NP induced oxidative damage and subsequent normalization of the animals' behaviour [19,21]. In short, excluding oxidative stress, melatonin's effect(s) on other HD associated alterations in the 3-NP model of HD is never well characterized. In the present study we have investigated the ability of melatonin to protect against 3-NP insult at the level of behaviour, neurotransmission, and neuronal morphology.

2. Materials and methods

2.1. Chemicals and reagents

3-NP, gelatin, Triton X -100, BSA, cresyl violet, EDTA, chloral hydrate, o-phthalaldehyde, DA hydrochloride, homovanillic acid (HVA), 3,4-dihydroxyphenyl acetic acid (DOPAC), serotonin (5-hydroxytryptamine, 5-HT), 5-hydroxyindole acetic acid (5-HIAA) and melatonin were purchased from Sigma–Aldrich (MA, USA). 3,3′-Diamino benzidine (DAB), acetonitrile, γ-amino butyric acid (GABA), glutamate and heptane sulphonic acid were procured from MP Biomedicals (CA, USA). All the other chemicals were of analytical grade and were purchased from SRL (Mumbai, India).

2.2. Animals and treatment

Animal experimentations were conducted in accordance with national guidelines on the 'Care and Use of Animals in Scientific Research', formed by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Animal Welfare Division, Ministry of Environment and Forests, Govt. of India. The protocol was permitted by the Animal Ethics Committee of CSIR-Indian Institute of Chemical Biology, Kolkata, India. Sprague Dawley male rats, 20–24 weeks old (350–400 g body weight) were housed under standard environments of temperature (22 ± 2 °C), humidity (60 ± 5%), and illumination (12-h light–dark cycle). They were provided with food and water *ad libitum*. Animals were always sacrificed in the mornings to avoid diurnal variations, if any. 3-NP was prepared freshly before each injection in normal saline (0.85% NaCl) and was adjusted to pH 7.4 by 5.0 N NaOH. Rats were treated with 20 mg/kg (i.p.) once daily, for 4 days. Melatonin was freshly prepared before each injection in minimal amount of ethanol. Rats were treated with 10 or 20 mg/kg body weight melatonin (i.p.), 1 h prior to 3-NP treatment; for four days. Control animals received equal amount of the vehicle, in the same volume.

2.3. Animal behaviour

Prior to any treatment, rats were weighed on every morning at the same time throughout the period of study. The method described by Klapdor et al. [33], and as standardized in our laboratory [17,34] was followed to quantify gait abnormalities. Briefly, rats were trained to walk on the platform 3–4 times until they started walking up the 1 meter length slanting platform leading to the dark housing with food pellet as reward. Foot impression was taken on white sheets; forelimbs and hind limbs were coloured with red and green non-toxic water colours, respectively. Stride length, stride width and foot length were measured from these foot impressions, manually.

Beam balance test was performed according to the protocol described by Wang et al. [35], and as standardized by us in the laboratory [34]. Animals were allowed to walk across a narrow wooden beam (2.0 cm in width, and 120 cm in length) elevated 65 cm above the ground. The beam was connected with a dark housing (22 cm × 15 cm × 18 cm) at the other end with food pellet inside as a reward. The animals were trained to walk on the narrow beam for 3 consecutive days. The time taken to cover the distance before and after the respective treatment regimen was noted down.

Swim test was carried out according to Haobam et al. [36], and further modified in the laboratory [37]. The animals were allowed to swim for a period of 10 min and the swim score was noted for every min. Animals were given swim scores as per the following scheme: 3: continuous swimming, 2: swimming with occasional floating, 1: more floating with occasional swimming with hind limbs and 0: hind parts sink with only the head floating.

For hang test animal was allowed to grip a steel wire (2 mm diameter) placed 70 cm above the ground and the duration of the period was noted [21].

For Morris water maze test we followed the protocol described by Vorhees et al. [38]. Briefly, a hidden platform was placed in the south-east coordinate of the water filled tank and the animal was placed on the north-west coordinate. The training was run for 90 s and it took 3 consecutive days for the animals to find the platform instantly. We monitored the time taken and the distance covered to reach the platform. We used a video tracking device and software (ANYmaze) provided with the instrument by SD instruments (CA, USA).

Animals were kept on a rotarod instrument (IITC Instruments, CA, USA) with a maximum speed of 15 rpm stating with 1 rpm, and the falling latency was monitored [21].

2.4. Determination of neurotransmitter levels and cresyl violet staining

After completion of the treatment period, striatum from one half of the brain was collected in 10 volumes of ice cold 0.1 N perchloric acid containing 0.01% EDTA, sonicated at 50 Hz for 30 s, centrifuged at 12,000 × *g* for 10 min and the supernatant was collected. Ten microlitre was injected into a HPLC system equipped with a Rheodyne injector, glassy carbon working electrode and Ag/AgCl reference electrode (Bioanalytical Systems Inc, USA). A