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Role of melatonin in mitigating nonylphenol-induced toxicity in frontal cortex and hippocampus of rat brain

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ABSTRACT

Nonylphenol (NP), an environmental endocrine disruptor mimics estrogen and is a potential toxicant both under *in vitro* and *in vivo* conditions. In this study, the effect of melatonin on NP- induced neurotoxicity and cognitive alteration was investigated in adult male Wistar rats. Melatonin supplementation has been known to protect cells from neurotoxic injury. The animals were divided into three groups namely, control (vehicle) which received olive oil orally and treated rats received NP (25 mg/kg, *per os*) thrice a week for 45 days while the third group i.e., NP + melatonin, animals were co-administered melatonin (10 mg/kg, *i.p.*) along with NP. On the 46th day, rats were assessed for anxiety, motor co-ordination, grip strength and cognitive performance using Morris water maze test and then sacrificed for biochemical and histopathological assays in brain tissues. Melatonin improved the behavioral performance in NP exposed group. The results showed that NP significantly decreased the activity of acetylcholine esterase (AchE), monoamine oxidase (MAO) and Na^+/K^+-ATPase, in rat brain tissue along with other enzymes of antioxidant milieu. The outcome of the study shows that NP, like other persistent endocrine disrupting pollutants, creates a potential risk of cognitive, neurochemical and histopathological perturbations as a result of environmental exposure. Taken together, our study demonstrates that melatonin is protective against NP-induced neurotoxicity.

1. Introduction

Most endocrine disrupting chemicals (EDCs) are manufactured industrially as a raw material for plastic packaging, cosmetics, detergents or paints, and as additives for lubricants, while a certain percentage of them are unwanted by-products and wastes (Mao et al., 2010). Humans are under continuous threat of exposure to many environmental xenobiotics and associated health hazards including EDCs. EDCs toxicity can potentially affect several organ systems including the pathophysiological role in central nervous system (CNS) and other neurological disorders. This peculiar ability to alter the neural transmission and the formation of neural networks, the EDCs are sometimes also referred to as neural-disrupting chemicals (Kakeyama et al., 2001). Certain EDCs like bisphenol A (BPA) and diethylstilbestrol (DES) have been shown to adversely affect synaptic plasticity (Ogiue-Ikeda et al., 2008).

Nonylphenol (NP) is one such example of a widely spread and uncontrolled distributed environmental non-ionic surfactant. NP is a lipophilic, ubiquitous persistent contaminant in both wildlife including aquatic environment and humans. The primary use of NP is as an intermediate in the manufacture of its ethoxylates. They are widely used in industrial applications as well as household and personal care products, including many plastics that have been in commerce for over 50 years. Products containing NP come from textile processing, pulp and paper processing, paints, resins and protective coatings, oil and gas recovery, steel manufacturing, pest control products and power generation sectors. NP's unique chemical structure makes it resistant to physical, chemical and biological degradation which may result in its longer retention in the environment. Its lipophilic nature can also lead to its cellular and tissue bioaccumulation (Mao et al., 2010). It is classified as EDC because of its structural similarity to the endogenous estrogens, e.g., 17-β-estradiol, and its ability to mimic or block hormonal effects (Jie et al., 2013).

Few reports have appeared about the negative effects of NP on CNS along with reproductive and immune systems. It can also have neurodevelopment changes in both animals and human beings. Some evidence exists on NP-induced CNS neurotoxicity (Jie et al., 2016). The neurotoxicity of NP could be due to oxidative stress

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via activation of mitochondrial apoptosis pathway and inflammatory signaling pathway, which influence the expression of apoptosis genes and inflammatory mediators. Zhang and Zhang, (2014) have reported that inducible nitric oxide synthase and cyclooxygenase-2 enzymes, which cause inflammation, are increased in mice brains due to chronic NP exposure leading to neurotoxicity. Uncontrolled chronic inflammation of the central nervous system can lead to neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. However, the mechanisms behind the NP neurotoxic and cognition impairing ability still needs detailed investigation. Analyzing behavioral change is a way to assess any abnormality in the neural function. The NP exposure has been correlated to the disruptive cognitive function and behavioral responses in fish, reptiles, and birds because the gonadal hormones have a direct regulatory effect on CNS development. Recently, reports have indicated potential CNS neurotoxicity induced by NP in various aquatic and rodent models (Jie et al., 2013). NP can penetrate blood-brain barrier (BBB) and has the potential to structurally mimic an endogenous hormone, estrogen or in some cases, block the effects of the same (Doerge et al., 2002; Litwa et al., 2014).

Recent studies have focused on the possible capacity of natural compounds and phytochemicals extracted from fruits, vegetables and beverages displaying protective abilities in various animal models of neurological disorders (Wu et al., 2010). Reduced glutathione (GSH) is one of the endogenous antioxidants and non-specific hydroxyl radical scavengers present in the brain. In addition, there are some exogenous antioxidants available in cells like flavonoids and vitamins which can also be supplemented if required (Valko et al., 2007). *N*-Acetyl-5-methoxytryptamine also known as melatonin is one such lipophilic hormone that is mainly produced and secreted at night by the pineal gland. Research studies have explored the several physiological properties of melatonin such as circadian rhythms regulation, free radical scavenging, improving immunity, analgesic and neuroprotection (Reiter et al., 2014). Generally, melatonin exerts both direct and indirect antioxidant protective actions both under *in vivo* and *in vitro* conditions (Reiter et al., 2003). It easily crosses morpho-physiological barriers like BBB, intracellular and sub-cellular barriers acting as a free radical scavenger (Reiter et al., 2009). The present study aimed to investigate the neurotoxic effects of chronic NP treatment on oxidative stress and cognitive alterations in male Wistar rats. Additionally, studies on protective action of melatonin co-administration against NP induced oxidative alterations in the brain tissue and associated neurobehavioral changes in exposed animals were also carried out.

2. Methodology

2.1. Chemicals

Acetylthiocholine iodide (ATC), benzylaminehydrochloride (BAHC), bovine serum albumin (BSA), 5,50-dithiobis (2-nitrobenzoic acid) (DTNB), oxidized glutathione (GSSG), reduced glutathione (GSH), reduced NADP(H), thiobarbituric acid (TBA) and xanthine were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). 1-amino-2-naphthol-4- sulphonic acid (ANSA), butylated hydroxyl taulene (BHT), 1- chloro-2,4-dinitrobenzene (CDNB), 2,4-dinitrophenylhydrazine (DNPH), epinephrine, EDTA, orthophosphoric acid (OPA), perchloric acid (PCA), sulphosalicylic acid, sodium azide and trichloroacetic acid (TCA) were purchased from Merck Limited (Mumbai, India). p-NFκB, p65 polyclonal and β-Actin mouse monoclonal antibody was obtained from Santa cruz Biotechnology, USA. Monoclonal GFAP antibody was purchased from Chemichon International, Temecula, CA and anti-rabbit IgG was purchased from Jackson Immuno Research Laboratories Inc. West Groove, PA, USA. Nonylphenol (NP) was obtained from Sigma–Aldrich chemicals Pvt. Ltd. USA.

2.2. Animals

The experimental protocol was approved by the Institutional Animal Ethics Committee (173/GO/Re/S/2000 CPCSEA). Male Wistar rats weighing 150–200 g, aged 5–6 weeks were maintained under standard conditions in an animal house (Central Animal House Facility, Hamdard University, New Delhi). Protocols were approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals. Animals were housed at room temperature with 12 h light and 12 h dark cycle. Food and drinking water was provided *ad libitum*. The animals were acclimatized for one week in the laboratory to adapt to the local environment and handling. During the maintenance period, each animal was taken out from the cage daily to record body weight, rectal temperatures using clinical digital thermometer.

2.3. Experimental design

NP was suspended in corn oil and administered via oral route in rats (25 mg/kg b. wt.) for 45 days. Neuroprotective agent, melatonin 10 mg/kg b. wt. suspended in distill water was administered via *i. p.* route. The animals were evaluated for neurobehavioral alterations employing several parameters at the end of the exposure regime. In addition to these parameters mentioned below, specific neurochemical, histopathological and immunohistochemical analysis were carried out for corroborating cognitive impairment results with probable neurotoxicity of NP.

2.4. Neurobehavioral paradigms

2.4.1. Morris water maze

The Morris water maze procedure was employed to assess cognitive function. The learning and memory capabilities of the rats were evaluated using water maze task (Rowe et al., 2007). The spatial learning and memory of animals were tested in an apparatus, which consisted of a circular water tank (132 cm diameter, 60 cm height) filled 40 cm with water($25 \pm 2^{\circ}$ C). A non-toxic paint was used to render the water opaque. The pool was divided into four equal quadrants, labeled north, south, east, and west. An escape platform (10 cm indiameter) was located 2 cm below the water surface in a constant position in one of four imaginary quadrants of the pool. The animal could use only distal visual cues (colored posters, pictures and a curtain) from within the testing room to locate the submerged platform. Latencies to locate the hidden platform during training and test trials (see procedures below) were recorded and analyzed using a computer-based tracking system (ANY-maze Video Tracking Software, USA). Before the training started, rats were allowed to swim freely in the pool for 60 s without platform. The animals were given 10 trials over 3 consecutive days (5 training trials on the first day followed by 4 trials on the second day and a single test trial on the last day at 20–30 min inter-trial interval) with the platform submerged. Trial duration was maximally 120 s; if the platform was not located the animal was guided to and had to remain on the platform for 20 s. Animals were placed into the pool facing the side wall. After each training trial, the rat was dried with a towel and allowed to remain in a cage. The time spent in the target quadrant indicated the degree of memory consolidation which had taken place after learning. The time and track taken to reach the platform was recorded by an overhead video camera. Latency to reach the platform was recorded up to a cutoff time of 120 s.