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Research Report

Melatonin promotes distal dendritic ramifications in layer II/III cortical pyramidal cells of rats exposed to toluene vapors

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ABSTRACT

We have previously shown that toluene inhalation produces significant impairments in the basilar dendritic outgrowth of pyramidal cortical cells. This neurotoxic effect was markedly inhibited by melatonin administration at a dose of 5 mg kg^{-1}. The present study was designed to determine whether toluene and melatonin equally affect all basilar dendritic segments or if a differential response exists between the segments. Twenty-eight male mice were weaned at postnatal day 21 (P21) and randomly assigned to either the control (C; $n=10$,) or toluene (T; $n=18$) group. Between P22-P32, male rats were placed into a glass chamber and exposed to either toluene vapors (5–000–6000 ppm) or clean air for 10 min a day. When toluene exposure ended (P32), animals were further assigned to the following experimental groups: (a) control/saline (C/S; $n=10$), (b) toluene/saline (T/S; $n=10$), or (c) toluene/melatonin 5 mg kg^{-1} (T/M; $n=8$). Melatonin or vehicle solutions were administered daily between P32 and P38. Forty-eight hours after the final toluene exposure, the animals were sacrificed, and the pyramidal cortical cells were stained using the Golgi–Cox–Sholl procedure. The number of basilar dendritic branches/order was counted using the centrifugal ordering method. The results indicate that (i) toluene inhalation significantly impairs both proximal and distal basilar dendritic ramifications (in the parietal and frontal/occipital cortices, respectively) and (ii) melatonin both protects neurons from toluene neurotoxicity in all cortical areas studied and increases the complexity of the dendritic tree above control values.

1. Introduction

In recent years, many publications have highlighted the protective role of melatonin (*N*-acetyl-5-methoxytryptamine) in the central nervous system (CNS). In many tissues (including the CNS), melatonin acts either via the nuclear hormone receptor family (primarily RZRβ) or through the membrane G-protein-coupled receptors MT1/MT2 (Hekmekekcioglu, 2006). In addition, melatonin can act in a receptor-independent manner as a major scavenger of both oxygen and nitrogen reactive species (Hibaoui et al., 2009). Because oxidative/nitrosative stress is a pathophysiological pathway that is common in many brain disorders (Barja and Herrero, 2000; Korkmaz et al., 2009), several preclinical studies have used this hormone in animal models of brain damage to evaluate its therapeutic potential. For example, it was found

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Abbreviations: CNS, central nervous system; P, postnatal day; C/S, control/saline; T/S, toluene/ saline; T/M, toluene/melatonin

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that melatonin can minimize behavioral and neural impairments induced by cerebral ischemia (Bouslama et al., 2007; Chen et al., 2009; Duan et al., 2006; García-Chávez et al., 2008; González-Burgos et al., 2007; Letechipía-Vallejo et al., 2007; Rennie et al., 2008;), hypoxia (Kaur et al., 2010), traumatic brain injury (Samantaray et al., 2009), and neurodegeneration (Lin et al., 2008; Weishaupt et al., 2006).

In addition, the chronic abuse of volatile solvents, especially toluene, is a common practice among children and adolescents at social risk—primarily for its anxiolytic effect and its role in activating the brain's reward pathways (Chouanière et al., 2002; Kurtzman et al., 2001). These psychotropic actions have led to the widespread recreational use of toluene and related volatile compounds (Chouanière et al., 2002). Chronic toluene exposure, either occupationally and/or intentionally, can lead to neurotoxic CNS damage such as brain atrophy, white matter impairments, multifocal hemodynamic changes and various neuropsychological deficits (Aydin et al., 2009; Deleu and Hanssens, 2000; Fornazzari et al., 1983; Hormes et al., 1986; Küçük et al., 2000; Kurtzman et al., 2001). Because the primary pathophysiological mechanism of toluene-induced neurotoxicity is oxidative/nitrosative stress (Burmistrov et al., 2001; Mattia et al., 1993), several studies have successfully demonstrated that melatonin can offset the neurotoxic effects of toluene. In fact, this indolamine can counteract toluene-induced lipid peroxidation and gliosis in the cerebral cortex, hippocampus and cerebellum (Baydas et al., 2003). Furthermore, we showed in a recent study that the total basilar dendritic length and branching impairment induced by toluene inhalation was significantly ameliorated by melatonin administration (Pascual et al., 2010). Because the basilar dendrites of cortical pyramidal cells follow a proximal-to-distal developmental time course, i.e., dendrites emerging directly from the soma (1st order dendrites) mature earlier than their 2nd order counterparts, and because distal dendrites appear to be more sensitive to various epigenetic cues (Hickmott and Ethel, 2006), it is possible that either the toxic effect of toluene or the protective-trophic-like action of melatonin can affect distinct dendritic loci. Because this phenomenon has not been studied, we took advantage of the multilevel centrifugal approach developed by Coleman and Riesen (1968) to evaluate the neurotoxic effect of toluene and the rescue effect of melatonin administration on the complexity of basilar dendritic branching of layer II/III frontal, parietal and occipital pyramidal cells (Fig. 1).

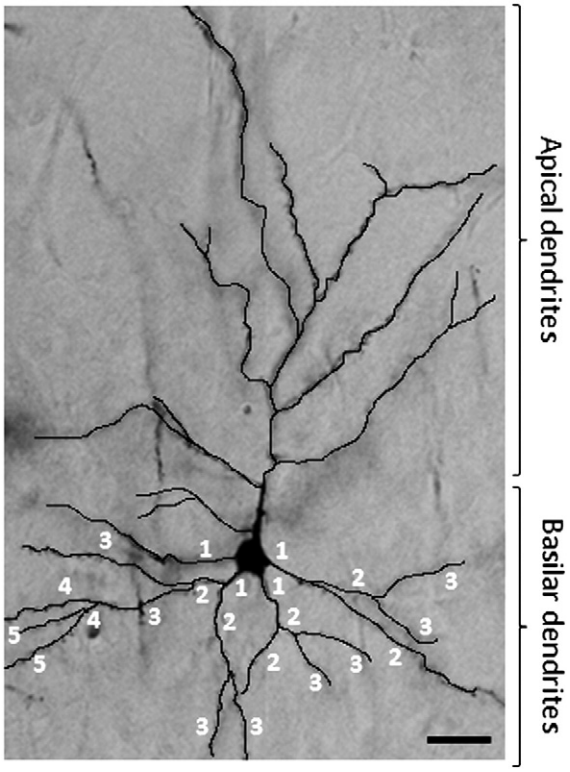

Fig. 1 – Basilar dendritic order quantification. Photomicrograph of a typical layer II/III Golgi-stained cortical pyramidal cell. The basal dendritic tree was redrawn to highlight the branches located outside the photographic plane. White numbers indicate dendritic orders (1: first-order dendrites arising from cell body; 2: second-order dendrites arising from first branching point, and so on). Bar = 50 μm.

2. Results

The number of dendritic branches per neuron in the 3rd, 4th, and 5th order of frontal pyramidal cells was significantly reduced in animals exposed to toluene fumes (Fig. 2; $^{*}p<0.05$, $^{**}p<0.01$; ANOVA). In contrast, toluene-exposed animals that were treated with 5 mg kg^{-1} melatonin showed a significant restorative effect on dendritic segments compared to the corresponding controls. In addition, as shown in the 3rd–6th orders, melatonin not only offset the deterioration caused by toluene but promoted dendritic branching above the level found in the control group. In contrast, toluene produced dendritic impairments only in proximal orders (2nd and 3rd) of parietal cortical neurons. Furthermore, while melatonin induced recovery following toluene, its trophic-like effect on dendritic branching was less (4th and 5th orders) than that observed in frontal neurons (Fig. 3; $^{*}p<0.05$, $^{**}p<0.01$; ANOVA). Finally, exposure to toluene altered occipital pyramidal cells only at their most distal dendritic branches. Despite rescue by melatonin administration, the branching overgrowth observed in the frontal and parietal cortices was not detected (Fig. 4; $^{**}p<0.01$; ANOVA). The neurotoxic effect of toluene and the ability of melatonin to recover/promote dendritic branching over control levels are shown in the representative photomicrographs accompanying the graphics. There were no differences in body weight between control and solvent/melatonin-exposed animals (Fig. 5).

3. Discussion

In the present study, we demonstrate that toluene inhalation in juvenile rats significantly impaired basilar dendritic tree ramifications and that melatonin not only protected neurons from toluene neurotoxicity but also increased dendritic complexity in two of the three regions studied (frontal and