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Chronic administration of a melatonin membrane receptor antagonist, luzindole, affects hippocampal neurogenesis without changes in hopelessness-like behavior in adult mice

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ABSTRACT

Melatonin is involved in the regulation of hippocampal neuronal development during adulthood. Emerging evidence indicates that exogenous melatonin acts during different events of the neurogenic process and exerts antidepressant-like behavior in rodents. Thus, melatonin might act through different mechanism, including acting as an antioxidant, interacting with intracellular proteins and/or activating membrane receptors. The melatonin membrane receptors (MMRs; Mt1/Mt2) are distributed throughout the hippocampus with an interesting localization in the hippocampal neurogenic microenvironment (niche), suggesting the involvement of these receptors in the beneficial effects of melatonin on hippocampal neurogenesis and behavior. In this study, we analyzed the participation of MMRs in the baseline neurogenesis in C57BL/6 mice. To this end, we used a pharmacological approach, administering luzindole (10 mg/kg) for 14 days. We observed a decrease in the absolute number of doublecortin-positive cells (49%) without changes in either the dendrite complexity of mature doublecortin-cells or the number of apoptotic cells (TUNEL). However, after the chronic administration of luzindole, cell proliferation (Ki67) significantly decreased (36%) with increasing (>100%) number of neural stem cells (NSCs; $GFAP^+/Sox2^+$) in the subgranular zone of the dentate gyrus of the hippocampus. In addition, luzindole did not affect hopelessness-like behavior in the forced swim test (FST) or changes in the novelty suppressed feeding test (NST) after 14 days of treatment either neuronal activation in the dentate gyrus after FST. These results suggest that the MMRs are involved in the effects of endogenous melatonin to mediate the transition from NSCs and proliferative cells to the following developmental stages implicated in the hippocampal neurogenic process of adult female C57BL/6 mice.

1. Introduction

Melatonin, the main product synthesized by the pineal gland (Reiter, 1991), acts as a regulator of adult hippocampal neurogenesis in rodents (Kim et al., 2004; Liu et al., 2013; Ramirez-Rodriguez et al., 2009; Rennie et al., 2009; Yoo et al., 2012). Additionally, it has been hypothesized that adult hippocampal neurogenesis facilitates adaptation to novel and complex situations and it is altered in preclinical models of depression (Kempermann, 2008).

Exogenous melatonin regulates cell proliferation, survival and neuronal maturation in the dentate gyrus (DG) of the hippocampus in different strains of mice and rats (i.e., (Liu et al., 2013; Ramirez-Rodriguez et al., 2009, 2011, 2012)) and in animal models of stress shows antidepressant-like effects (i.e., (Crupi et al., 2010, 2011; Kim et al., 2004; Ramirez-Rodriguez et al., 2009)). Additionally, melatonin rescued the functional and morphological deficits in an animal model of Down's syndrome (Corrales et al., 2014); and melatonin attenuates the observed decrease in immature neurons caused by cranial irradiation (Manda and Reiter, 2010; Manda et al., 2009), and regulates neurogenesis and behavior after mild focal cerebral ischemia in mice (Kilic et al., 2008).

In a previous study, we showed that the chronic administration of melatonin promoted the survival of newborn neurons, increased the number and dendrite complexity of doublecortin (DCX)-

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associated cells and prevented the depressive behavior of female C57BL/6 mice in the Porsolt's forced swim test (FST) (Ramirez-Rodriguez et al., 2009, 2011).

The mechanisms involved in the beneficial effects of exogenous melatonin on hippocampal neurogenesis under physiological or non-physiological conditions (i.e., stress) might include the activation of melatonin membrane receptors (MMRs) (Dubocovich, 1988, 2007; Dubocovich and Markowska, 2005), antioxidant activity (by melatonin or its metabolites i.e. AFMK) (Manda et al., 2008; Tan et al., 2002) and/or interactions with intracellular proteins, such as calmodulin, protein kinase C and potentially calretinin (Benitez-King and Anton-Tay, 1993; Benitez-King et al., 2001; Ramirez-Rodriguez et al., 2014a).

Regarding the participation of MMRs in the effects of melatonin, several studies have used pharmacological approaches involving MMRs antagonists (i.e., (Dubocovich et al., 1990; Sumaya et al., 2005)). Thus, the MMRs antagonist luzindole has been widely used to identify the relevance of MMRs in melatonin activity (Boutin et al., 2005). Luzindole exhibits high selectivity and affinity for MMRs determined through competition of melatonin receptor for 2-[^{125}I]-iodomelatonin binding to lysates of COS-7 cells expressing MMRs (Mt1: Ki = 158 nM; Mt2: Ki = 10.2 nM; Mt1/Mt2 = 15.5) (Dubocovich, 1988; Dubocovich et al., 1997, 1998; Boutin et al., 2005). These receptors are found at various locations in the mammalian central nervous system (CNS) (Dubocovich, 1988; Boutin et al., 2005). Interestingly, the acute administration of luzindole induced antidepressant-like effects through the blockade of one of the MMRs (Mt2, also referred to as MTnr1b or Mel1b) in C3H mice (Dubocovich et al., 1990; Sumaya et al., 2005). However, Mt1KO C57BL/6 mice showed increased hopelessness-like behavior in the FST (Adamah-Biassi et al., 2014).

Recently, the expression of MMRs was mapped in the male rat brain, and both, Mt1/Mt2, receptors were identified in the DG (Lacoste et al., 2015). Similarly, Mt1/Mt2 have been reported in neural precursor cells obtained from adult rats (Tocharus et al., 2014). Moreover, the Mt2 receptor is expressed in the brain of adult male C57BL/6 mice; and according to the Allen brain atlas, Mt2 expression is concentrated in, but not limited to, the neurogenic niche of the hippocampus (www.brain-map-org; Mtnr1b-RP_050331_04-A05-Saggital). In addition, extracts of dissected-DG showed the expression of mRNAs for Mt1/Mt2 receptors (Ramirez-Rodriguez et al., 2009). Additionally, isolated hippocampal neural precursor cells, comprising neural stem and progenitor cells, from adult female C57BL/6 mice also showed Mt1/Mt2 mRNAs after culture under proliferation conditions or after the induction of differentiation (Ramirez-Rodriguez et al., 2009).

Thus, we hypothesized that the MMRs might be involved in the regulation of hippocampal neurogenesis and hopelessness behavior, tested in the FST, through endogenous melatonin in adult female C57BL/6 mice. This strain of mice synthesizes melatonin at lower plasma levels than other strains of mice. In these inbred mice, high performance liquid chromatography analysis and radioimmunoassay confirmed a short-term peak in melatonin expression in the plasma during the middle of the dark period. Although low levels of melatonin are found in the pineal and blood of C57BL/6 mice, it has been indicated that this small amount of the indole would be sufficient to physiologically activate MMRs (Conti and Maestroni, 1996; Vivien-Roels et al., 1998; Dubocovich et al., 2010; Gomez-Corvera et al., 2009; Ramirez-Rodriguez et al., 2009).

Thus, in the present study, as a pharmacological approach, we chronically administered luzindole for 14 days to evaluate the relevance of MMRs on hippocampal neurogenesis, hopelessness-like behavior in the FST and neuronal activation in the DG after FST.

2. Materials and methods

2.1. Animals

Female C57BL/6 mice were obtained from Harlan (México City, México). Animals were housed in standard laboratory cages under 12-h light/12-h dark cycles at 23 ± 1 °C in the animal facilities of the National Institute of Psychiatry until the rodents reached 8 weeks old. The light/dark cycle corresponded to the timing of lights on (Zeitgeber time 0; ZT0) at 0700 h and lights off (Zeitgeber time 12; ZT12) at 1900 h, respectively. The mice had access to food and water ad libitum. All institutional and legal regulations regarding animal ethics and handling were followed for the in vivo experiments (Ethical Committee Approval: IACUC SIC092025). The rational for using female C57BL/6 mice was based on our previous studies in which melatonin acted as a regulator of adult hippocampal neurogenesis producing an antidepressant like effect in the FST (Ramirez-Rodriguez et al., 2009, 2011).

2.2. Luzindole administration

The mice were treated with luzindole (Sigma, Naucalpan, Estado de México, México) at the beginning of the dark phase in the light–dark cycle, and the antagonist was daily administered (10 mg/kg of body weight, BW; intraperitoneal) for 14 consecutive days. The dose administered was selected based on previous studies, in which doses ranging between 10 and 30 mg/kg showed antidepressant-like effects in mice (Dubocovich et al., 1990). Mice of control group received vehicle (1% Tween 80/1% ethanol in sterilized saline solution; 0.9% NaCl; Pisa, Guadalajara, Jalisco, México) (Dubocovich et al., 1990). The day after the last vehicle or luzindole injection, the mice were through an overdose of ketamine and transcardially perfused with 4% para-formaldehyde in 0.1 M of phosphate buffer (PB. pH 7.4). The brains were transferred to 30% sucrose in PB.

2.3. Tissue processing immunohistochemistry

The brains were cut into 40-μm coronal sections with a sliding microtome (Leica, Buffalo Grove, IL, USA) and stored at 4 °C in cryoprotectant solution (25% ethylene glycol and 25% glycerin in 0.05 M phosphate buffer). The sections were stained following the free-floating immunohistochemistry method (Ramirez-Rodriguez et al., 2011, 2012).

2.4. Immunohistochemistry and quantification of Ki67- or doublecortin cells

Brain coronal sections were incubated with primary antibodies for the immunodetection of Ki67- or DCX-positive cells in the DG. The antibodies used were rabbit anti-Ki67 (Abcam, San Francisco, CA, USA) and goat anti-DCX (Santa Cruz Biotech, Santa Cruz, CA, USA), and the results were visualized using the peroxidase method (Ramirez-Rodriguez et al., 2011, 2012).

The number of Ki67- or DCX-positive cells was determined every 6th section from all animals (Ramirez-Rodriguez et al., 2011, 2012). Cells positive for Ki67 were counted exhaustively using a 40× objective throughout the rostro–caudal extent of the sugranular zone, whereas DCX was counted in both subgranular zone (SGZ) and granular cell layer (GCL). Counting was performed as previously described using the modified optical dissector method under bright-light microscopy (Leica, Buffalo Grove, IL, USA). The cells appearing in the uppermost focal plane were excluded to avoid over-sampling (Kempermann et al., 2003). The resulting numbers were multiplied by six to obtain the estimated total