Melatonin Increases Interleukin-1β and Decreases Tumor Necrosis Factor Alpha in the Brain of Mice Infected with the Venezuelan Equine Encephalomyelitis Virus

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The effect of melatonin (MLT) on the brain levels of tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β) in Venezuelan equine encephalomyelitis (VEE) virus infection was determined. Brain homogenates from mice inoculated with 10 LD50 of VEE virus, untreated or treated with 500 µg MLT/kg body weight were assayed by ELISA to measure the levels of TNF-α and IL-1β. MLT was injected daily starting 3 days before and continuing to 7 days after virus inoculation. Infected mice treated with MLT showed decreased levels of TNF-α when compared to the untreated infected mice on days 1, 3, 4, and 5 postinoculation (P < 0.001). In contrast, IL-1β levels increased from days 1 to 5 in the infected mice treated with MLT when compared with the untreated infected animals (P < 0.01). The results suggest that the protective effect of MLT on the VEE virus infection could be due, among other factors, to a decrease in TNF-α synthesis along with an increase in the production of IL-1β.

KEY WORDS: Melatonin; Venezuelan equine encephalomyelitis virus; tumor necrosis factor-alpha; interleukin-1β.

INTRODUCTION

Melatonin (MLT, N-acetyl-5-methoxytryptamine) appears to be involved in synchronizing the circadian and seasonal timing of several physiological and behavioral processes (1,2), and it seems to be a powerful hydroxy radical scavenger that provides on-site protection against oxidative damage to cell components (3).

Because of its high solubility on lipids and high degree of hydrophilicity (4), this hormone not only crosses the cell membrane to enter the cytosol, but it also has access to every subcellular compartment without the help of a carrier molecule (3). MLT plays an important immunoregulatory role in both physiological and physiopathological conditions (5,6). By binding to T-helper cells, MLT gives rise to a series of events leading to an increase in immune response (7) that seems to be mediated, at least in part, by endogenous opioid peptides (8), and T-cell–derived cytokines (9). In fact, when chronically injected into young mice or those immunodepressed by aging or cyclophosphamide, MLT was able to enhance the antibody response to a T-dependent antigen (10).

A protective effect of MLT was reported in mice infected with the Semliki Forest virus (SFV) and in stressed mice infected with the attenuated noninvasive

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West Nile virus (WN-25) (11). It has been shown that MLT protects mice infected with the highly lethal Venezuelan equine encephalomyelitis (VEE) virus by postponing the onset of the disease and death by several days (12). It also prolonged survival of immunodepressed mice infected with the VEE virus (13). The antiviral effect of MLT on VEE infection could be mediated by the stimulation of the immune system, which induces an alteration in the pattern of production of some cytokines that, in turn, would modulate the mechanisms of antiviral immunity in the early stages of VEE infection. During this infection, coinciding with the highest titers of virus in the brain, the central nervous system (CNS) shows an increment of the cellular infiltrate, with areas of cortical necrosis and marked macrophagic activity (14).

Tumor necrosis factor-alpha (TNF-α) is a powerful inflammatory cytokine produced in neurons in the normal murine brain (15). It is a 17-kD peptide synthesized by a wide variety of cells during host responses to microbial infections. Activated macrophages are the major cellular source for TNF-α, but other cell types such as endothelial cells, microglia, and astrocytes can be stimulated to secrete TNF-α (16).

Interleukin-1β (IL-1β) is a 17-kD peptide produced by astrocytes in the CNS (17) and can enter the brain via a specific carrier-mediated blood-brain barrier (BBB) transport system, suggesting that systemic inflammation resulting in IL-1β production could have repercussions in the CNS (18). For example, sickness behavior, which refers to a coordinated set of behavioral changes that develop in sick individuals during the course of an infection, is due to the brain effects of proinflammatory cytokines such as IL-1β (19). On the other hand, IL-1β is released during the acute phase immune response and can directly stimulate the Release of Corticotrophin-releasing hormone and thus induce the hypothalamic-pituitary-adrenal axis hyperactivity found in major depression and postviral depression (20). It has also been found that IL-1β stimulates the release of several neurotransmitters, including norepinephrine, dopamine, 5-hydroxytryptophan, and nitric oxide; it induces alterations in the electrophysiological properties of neurons and modulates autonomic function, including body temperature and cardiovascular regulation (21). Therefore, IL-1β secreted either by intrinsic brain cells or by infiltrating inflammatory cells, can result in neuronal dysfunction by affecting neurotransmitter synthesis, the influx of ions, or nitric oxide production (22).

This study was designed to test the effect of MLT on the levels of TNF-α and IL-1β in the brain of mice infected with the VEE virus.

EXPERIMENTAL PROCEDURE

Male albino mice (NMRI-IVIC strain) from the Venezuelan Institute for Scientific Research, weighing 25–30 g and fed ad libitum with laboratory chow and tap water, were maintained in a room with controlled temperature (24°C) under a 12-h light/dark cycle.

The VEE virus stock used for experiments was prepared in Vero cells and contained $6.8 \times 10^7$ plaque-forming units per ml (PFU/ml). The mice were inoculated intraperitoneally with 0.05 ml containing 10 LD$_{50}$ of the Guajira strain of VEE virus suspended in 0.4% bovine albumin borate–buffered saline solution (BABS). Viral infectivity titers were determined by plaque assay in chicken embryo fibroblasts (23).

Melatonin (Research Biochemical International, Natick MA, USA) was diluted in phosphate-buffered saline (PBS) and injected (500 μg/kg body weight) daily subcutaneously, 2 h before darkness, starting 3 days before and continuing to 7 days after virus inoculation (11). Control animals received PBS instead of MLT. One, three, four, and five days postinfection, mice were anesthetized and perfused intracardially with a cold solution of PBS. The brains were quickly removed and homogenized in 0.05 M Tris-HCl buffer pH 8.0 (20% w/v) and then centrifuged (7000 g) at 4°C for 10 min. The quantitative determinations of the murine cytokines TNF-α and IL-1β in the supernatants were based on a solid-phase ELISA capture system developed by Amersham International plc (Biotrack™).

Data are expressed as mean ± SEM of four experiments and were evaluated by ANOVA and the Bonferroni’s multiple comparisons test where appropriate. Differences were considered statistically significant when P < 0.05.

RESULTS

As shown in Fig. 1, VEE virus infection produced a striking increase in brain TNF-α levels from days 1 to 5 postinfection, reaching a maximum concentration of 1334.9 ± 60.1 pg/g of tissue on the fifth day, 30 times higher than the levels detected in control mice (44.8 ± 4.0) (P < 0.001).

Infected mice, when treated with 500 μg MLT/kg body weight showed decreased levels of TNF-α when

![Fig. 1. Levels of TNF-α in brain homogenates of mice infected with the Venezuelan equine encephalomyelitis (VEE) virus and treated with melatonin (MLT, 500 μg/kg body weight), at different postinfection stages.](image-url)