Stimulation of murine splenocytes by melatonin and methoxytryptamine

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Summary. Male C 57 mice kept under a 14:10 (L:D) photoperiod received vehicle (VEH), melatonin (MEL) and methoxytryptamine (MTA) in the drinking water for 2 weeks. Splenocytes from MEL-treated mice showed an augmented mitogenic response to concanavalin A and lipopolysaccharide (LPS) while splenocytes from MTA-treated mice demonstrated an enhanced mitogenic response to LPS when compared to the VEH-treated control. Splenocytes from MEL-treated and MTA-treated mice also produced higher levels of gamma interferon and interleukin-2. Lymphokines prepared from splenocytes of MEL-treated mice stimulated peritoneal macrophages to produce more nitrite than those from splenocytes of MTA-treated and control mice, suggesting that MEL had a stronger stimulating effect on the lymphocytes than MTA. Understimulation of lymphokines from MEL-treated mice, peritoneal macrophages from MTA-treated mice produced a greater inhibition of the growth of murine mastocytoma P 815 cells than that produced by macrophages from control and MEL-treated mice, suggesting that MTA was more potent than MEL in rendering the macrophages responsive to lymphokines. The results point to immunostimulatory actions of the pineal indoles MEL and MTA.

Keywords: Melatonin, methoxytryptamine, mouse splenocytes, immunostimulation.

Introduction

The pineal gland secretes a number of indoles including melatonin and methoxytryptamine. Pawlikowski (1988) reported that melatonin at concentrations of 10⁻⁴⁻⁻¹⁰⁻⁸ M exerted no significant influence on [³H]thymidine incorporation by spleen lymphocytes. Arzt et al. (1988) found that only at a concentration in the range of 10⁻³⁻¹⁰⁻⁴ M did melatonin inhibit proliferation of human peripheral blood T lymphocytes optimally stimulated by phytohem-
agglutinin. However, proliferation of human peripheral blood T lymphocytes stimulated suboptimally by phytohemagglutinin was inhibited by melatonin at a concentration in the range of $10^{-3}$ to $10^{-7}$ M.

Maestroni et al. (1986) found that melatonin injected into mice was able to antagonize the depression of antibody production by corticosterone in vivo and reverse the suppression of humoral response and autologous mixed lymphocyte reaction by propranolol (β-antagonist) and p-chlorophenylalanine (a depleter of serotonin in the central nervous system) (Sanders-Bush et al., 1974). Naltrexone, an opioid receptor blocker, was able to antagonize the enhancing effect of melatonin on primary antibody response in vivo, suggesting that melatonin enhanced the antibody response via an opiatergic mechanism. Maestroni et al. (1988) also noticed that naltrexone antagonized the counteracting effect of melatonin on immunosuppression induced by acute stress. Evening melatonin injections prevented paralysis and death of mice induced by sublethal doses of encephalomyocarditis virus after acute stress (Maestroni et al., 1988). Administration of melatonin into mice was able to enhance the antibody-dependent cellular cytotoxicity of leukocyte effector cells. The enhancement was not inhibited by the opioid antagonist naloxone (Maestroni, 1991).

The few earlier investigations about the immunomodulatory effect of pineal indoles were concentrated on serotonin and melatonin. The present study compared the immunomodulatory effects of melatonin with those of methoxytryptamine in view of the fact that both indoles were capable of inhibiting spermatogenesis (Ooi and Ng, 1989) and androgen production (Ng and Lo, 1988).

**Materials and methods**

**Chemicals**

Melatonin (MEL), methoxytryptamine (MTA), lipopolysaccharide (LPS) from Escherichia coli, and Concanavalin A (Con A) were obtained from Sigma Chemical Co. U.S.A.

**Culture media**

Dulbecco's modified Eagle's medium without phenol red (DMEM, D 2902), Dulbecco's phosphate buffered saline (PBS, D 5652), Hank's balanced salts (HBSS, H 2387) and RPMI-1640 (R 6504) were purchased from Sigma Co., U.S.A. RPMI and DMEM were supplemented with 10% fetal bovine serum (Gibco, U.S.A.), streptomycin sulfate (100 μg/ml) and penicillin G (100 IU/ml).

**Animals**

Male inbred C57BL/6 mice, aged 8-12 weeks old, were used in this study. The animals were kept at 21 ± 2°C under a 14/10-light/dark cycle and maintained on standard rodent chow. One group received 100 μg MEL/ml drinking water, another group received 100 μg MTA/ml drinking water, while the third group receiving 0.38% alcohol in drinking water served as the control. The treatment lasted for 2 weeks.

**Preparation of splenocytes for assay**

Animals were killed by cervical dislocation and spleens were aseptically excised. Splenocytes were obtained by pressing through a 100-mesh stainless steel sieve, washed, counted and resuspended at a concentration of $1 \times 10^7$ cells/ml RPMI medium.