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Melatonin receptor subtypes Mel1a and Mel1c but not Mel1b are associated with monochromatic light-induced B-lymphocyte proliferation in broilers

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ABSTRACT

This study determined the effects of melatonin (MEL) and its receptors on monochromatic light-induced bursal B-lymphocyte proliferation in broiler chickens. In vivo, green light (GL) enhanced the proliferation of B lymphocytes in bursas by 16.49% to 30.83% and the expression of MEL receptor subtypes 1a (*Mel1a*), *Mel1b*, and *Mel1c* receptors in bursas by 6.91% to 366.98% than other light colors. However, pinealectomy reduced these parameters and eliminated the differences between GL and other light groups. In vitro, the MEL-induced bursal B-lymphocyte proliferation was most suppressed by prazosin ($P = 0.001$, selective Mel1c antagonist), followed by luzindole ($P = 0.022$, nonselective Mel1a/Mel1b antagonist), but not by 4-phenyl-2-propionamideotetralin ($P = 0.144$, selective Mel1b antagonist). Similarly, dibutyryl-cyclic adenosine monophosphate (cAMP; analog of cAMP; $P = 0.017$) but not 8-(4-chloro-phenylthio)-2′-O-methyladenosine-3′,5′-cyclic monophosphate ($P = 0.736$; activator of exchange protein directly activated by cAMP) significantly inhibited bursal B-lymphocyte proliferation. These results suggest that MEL mediates GL-induced bursal B-lymphocyte proliferation through Mel1c and Mel1a receptors but not Mel1b receptors by activating the cAMP/protein kinase A pathway.

1. Introduction

The bursa of Fabricius is a primary immune organ that plays a critical role in the development of avian B lymphocytes. Its development is influenced by external factors, such as high doses of dietary vanadium [1] and heat stress [2]. However, few studies have evaluated the influence of light on the bursas of chickens. The regimen and wavelength of light contribute to cellular and humoral immune responses in birds [3–5]. Our previous study indicated that green light (GL; 560 nm) better enhances spleen T-lymphocyte proliferation in chickens at an early age than other wavelengths [6]. However, the effect and mechanism of action of monochromatic light on B lymphocytes are still unclear.

The photoperiod and wavelength of light can influence the secretion of melatonin (MEL) [7,8]. In addition, red light (RL) and blue light (BL) suppress the activity of arylalkylamine N-acetyltransferase (a rate-limiting enzyme associated with the synthesis of MEL) in chick pineal cells more efficiently than GL [9]. These findings suggest that MEL may transmit external light signals to intracellular molecules to influence intracellular events. Melatonin enhances immune function in mice [10] and Japanese quail [11]. The proliferation of chicken splenocyte (phytohemagglutinin [PHA]-stimulated) can be inhibited by MEL in vitro [12], but the splenocyte proliferation (concanavalin A- or lipopolysaccharide [LPS]-induced) in broilers [4] and mice [13–15] can be promoted by MEL.

Within the immune system, MEL acts via membrane G protein-coupled receptors [16], nuclear orphan receptors [17,18], or both, followed by the modulation of cytokine production [19]. The known MEL membrane receptors are

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Mel1a, Mel1b, and Mel1c [20]. Melatonin receptor 1 (MT1; Mel1a) or MT2 (Mel1b) is widely expressed in rat [7], squirrel [7], porcine [21], and *Perdicula asiatica* [22], whereas Mel1c is only present in fish, chicken, and *Xenopus* [23]. There is no consensus on the specific subtype of MEL receptor that mediates the effect of MEL on lymphocytes. Melatonin may act on Jurkat T lymphocytes via MT1 [19] or may enhance mouse spleen T-lymphocyte proliferation via MT2 [15]. The specific subtype of MEL receptor involved in the immunoregulatory effects of MEL in broilers is unknown.

We hypothesize that various wavelengths of monochromatic light influence the proliferation of bursal B lymphocytes in broilers at an early age. Thus, our objectives were to identify the role of MEL in facilitating the effect of monochromatic light on bursas and to further identify the specific subtype of MEL receptor that mediates the immune enhancement by MEL of bursal B lymphocytes of broilers at an early age.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the Animal Welfare Committee of the Agricultural Research Organization, China Agricultural University (Beijing). A total of 125 post-hatching day (P) 0 Arbor Acre male broilers (Beijing Huadu Breeding Co, Beijing, China) were used and allocated into 4 separate color light rooms, white light (WL; 400–760 nm), RL (660 nm), GL (560 nm), and BL (480 nm) room, by using a light-emitting diode (Hongli Tronic Co, Guangzhou, China) system for 2 wk [24]. All light sources were equalized to an illuminance of 15 ± 0.3 lux at bird-head level, with a light period of 23 h daily (light off at 11:00 PM) [24]. Each room contained 6 replicate cells except GL control rooms that contained 7 cells (5 birds per cell in all rooms) at a density of 11.5 birds/m^2, which included pinealectomy (2 cells, 10 birds), sham operation (2 cells, 10 birds), and intact group (2 cells, 10 birds). In addition, another 5 intact birds in the GL group were used for assay in vitro. The pinealectomy and sham operation were performed on each light-treatment group at P3. The ambient temperature was maintained at approximately 32°C in the first week and at 25°C in the second week, and the relative humidity was maintained at 60%. Chicks had ad libitum access to feed and water. The diet was formulated to meet or exceed the nutrient recommendations for poultry of the National Research Council (1994).

2.2. Sampling

The samplings were analyzed by the methods of lymphocyte proliferation assay, immunohistochemical staining, Western blot analysis, and RT-PCR at P14. Five birds from 1 cell per treatment (intact, pinealectomy, and sham-operation group) per room were selected, and whole bursas of Fabricius were aseptically removed to prepare single-cell suspension for lymphocyte proliferation assay. The remaining 5 birds in another cell per treatment per room were selected, and whole bursas of Fabricius were removed, which were divided into 3 parts for immunohistochemistry, Western blot analysis, and RT-PCR. One-third of the bursa was fixed in 4% paraformaldehyde in 0.1 *M* PBS (pH 7.4, 4°C) for 48 h; another one-third of the bursa was lysed in RIPA buffer; the remaining one-third of the bursa was used for total RNA extraction by Trizol. In addition, the other 5 intact birds in the GL group were used for determining the subtype of MEL receptor and possible signaling pathway in vitro.

2.3. Lymphocyte proliferation assay

Single-cell suspensions were filtered by a tissue sieve (200 mesh per 2.5 cm) and washed 3 times with RPMI 1640 medium (GibcoBRL, Grand Island, NY, USA). Finally, lymphocytes were suspended in 2 mL of RPMI 1640 complete medium. Cell viability (>98%) was estimated by trypan blue exclusion test, and final suspensions were adjusted to 10^7 cells/mL with RPMI 1640 complete medium. Bursal lymphocytes (10^6 cells/well) were cultured in triplicate in 96-well microtiter plates (Costar 3599; Corning Inc, Corning, NY, USA), immediately after which we added LPS (25 μg/mL; Sigma, St. Louis, MO, USA) and cultured the cells in an incubator at 41°C and 5% CO_2 for 48 h. Control cells were incubated with RPMI 1640 complete medium alone. Proliferation was measured with the methyl thiazolyl tetrazolium (Sigma) assay [25–28]. The results are expressed as the stimulation index (SI), which was calculated for each sample as the OD value (570 nm) for cells with stimulation divided by the OD value (570 nm) for cells without stimulation.

Beyond that, 5 whole bursas in the GL intact group (broilers under GL without pinealectomy and sham-operation) were prepared for single-cell suspensions as described in "Lymphocyte proliferation assay". One-half of single-cell suspensions in each bursa were respectively preincubated with 10 μ*M* luzindole (nonselective Mel1a/Mel1b antagonist; sc-202700; Santa Cruz Biotechnology Inc, Dallas, TX, USA), 0.1 μ*M* 4-phenyl-2-propionamideotetralin (4P-PDOT; selective Mel1b antagonist; Tocris Bioscience, Bristol, UK), or 0.1 μ*M* prazosin (selective Mel1c antagonist; sc-204858; Santa Cruz Biotech-nology Inc) for 30 min, followed by the addition of LPS (25 μg/mL) and MEL (250 pg/mL; 63610; Sigma) and incubation for 48 h. The other one-half of single-cell suspensions in each bursa were incubated with MEL, 10 μ*M* dibutyryl-cyclic adenosine monophosphate (dcAMP; analog of cAMP; sc-201567; Santa Cruz Biotechnology Inc) or 25 μ*M* 8-(4-chloro-phenylthio)-2′-O-methyladenosine-3′,5′-cyclic monophosphate (8Me-cAMP; activator of exchange protein directly activated by cAMP [Epac]; C8988; Sigma) and simultaneously stimulated by LPS for 48 h. Control cells were incubated alone in RPMI medium, MEL, luzindole, 4P-PDOT, prazosin, or 0.01% ethanol/dimethylsulfoxide. All the dose of antagonist, analog, and activator was decided by preliminary experiments. The results from methyl thiazolyl tetrazolium assays were analyzed to test for differences due to treatment and were expressed as the SI.

2.4. Immunohistochemical staining

Paraffin sections (5 μm in thickness) were prepared and immunohistochemically stained for Mel1a, Mel1b, and Mel1c. Sections were incubated overnight at 4°C with