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Effects of light-emitting diode spectra on the vertebrate ancient long opsin and gonadotropin hormone in the goldfish *Carassius auratus*

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ABSTRACT

We determined the molecular mechanism underlying the environmental (photoperiodic) regulation of sexual maturation in fish, we examined the expression of sexual maturation-related hormones and vertebrate ancient long opsin (VAL-opsin) in goldfish (*Carassius auratus*) exposed to different light spectra (red and green light-emitting diodes). We further evaluated the effect of exogenous gonadotropin hormone (GTH) on the expression of VAL-opsin under different light conditions. Our results demonstrated that the expression of GTHs was higher in the fish exposed to green light, and VAL-opsin levels were increased in the fish receiving GTH injection. Therefore, we have uncovered a molecular mechanism underlying the environmental (light)-induced trigger for sexual maturation: VAL-opsin is activated by green light and GTH, which promotes the expression of sexual maturation genes.

1. Introduction

Light directly or indirectly affects the circadian rhythm, growth, and sexual maturation of fish [1–3]. As the light passes through the retina, it sends a signal to the neurons in the brain [4]. To date, studies on the biological mechanisms associated with light have generally focused on the visual light path (retina); however, little is known about the effects of light on non-visual pathways (i.e., the deep brain, pineal complex, and skin), despite their important roles in animal behavior and physiology [5,6].

Vertebrates, including fish and birds, have photoreceptor cells in the retina (rods, cones and ganglion cells) as well as in various organs such as the pineal complex, deep brain, and skin, which regulate activation of the hypothalamus–pituitary–gonad (HPG) axis [3,7,8]. Initial studies conducted in birds identified the role of opsin genes in promoting maturation through photoperiodic control, demonstrating the important role of deep-brain photoreceptors in the hypothalamus, such as opsin-like proteins, in regulation of the HPG axis [7,9,10].

The pioneering study on vertebrate deep-brain photoreception demonstrated that a light-induced change in skin color of the European minnow (*Phoxinus laevis*) is not abolished by removal of the eyes and pineal complex, and the light sensitivity was instead ascribed to the “deep-brain photoreceptor” located at the ependyma of the diencephalic ventricle [11]. The involvement of deep-brain photoreceptors in the photoperiodic response of gonadal development was also suggested in channel catfish (*Ictalurus punctatus*) [12–14].

To date, several opsins, including rhodopsin, cone-like opsin, vertebrate ancient (VA) opsin, VA-long (VAL) opsin, and melanopsin, have been found in the brains of fishes [15–19]. Among these, VA-opsin and VAL-opsin share a common core sequence in the membrane-spanning domains, although VAL-opsin has a much longer C-terminal tail than that of VA-opsin. Functional reconstitution experiments on the recombinant proteins showed that VAL-opsin bound with 11-cis-retinal is a green-sensitive pigment (λmax ~ 500 nm), whereas VA-opsin exhibited no photosensitivity even in the presence of 11-cis-retinal [15]. Furthermore, it has been demonstrated that the deep-brain stimulator VAL-opsin is closely related to maturation in birds [9].

Sexual development and maturation in teleosts are regulated by various sex hormones in the HPG axis, including gonadotropin-releasing hormone (GnRH), gonadotropin hormone (GTH), and

Abbreviations: FSH, follicle-stimulating hormone; GTH, gonadotropin hormone; HPG, hypothalamus–pituitary–gonad; LH, luteinizing hormone; LED, light-emitting diode; VAL-opsin, vertebrate ancient long opsin.

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steroid hormones, and are regulated elsewhere by neuroendocrine materials and gonadal hormones [20]. In particular, GTHs are pituitary hormones that are secreted by GnRH stimulation, and are reported to play important roles in the regulation of gonadal development and sexual differentiation and stimulate spawning times and steroid hormone regulation in vertebrates, including fish [21]. In general, in fish, follicle-stimulating hormone (FSH) is involved in early gametogenesis, vitellogenin synthesis, and spermatogenesis, whereas luteinizing hormone (LH) is known to regulate final gonad maturation, ovulation, ejaculation, and steroid hormone synthesis [22,23].

Light induce or inhibit the photoreceptors in organisms, thereby influence the physiological processes [24] and sexual maturation by strongly affecting neuroendocrine control and the HPG axis [25,26]. Recent research has provided novel insights into the effect of light on fish maturation [3,27–29]; however, understanding of the effect of the various wavelengths of light on photoreceptor activation and photoreceptor-related maturation remains incomplete.

So, a light-emitting diode (LED) has the dual advantage of being able to emit light within a specific wavelength range with easily adjustable sensitivity. Thus, an LED is highly effective in light-related research [30,31]. In addition, Migaud et al. [2] showed that most of the light energy is wasted in the long wavelengths such as red light, which is rapidly absorbed by water molecules. Thus, a fish can generally detect short wavelengths such as green light better than longer wavelengths such as red light. However, studies on the relationship between VAL-opsin, a green-sensitive photoreceptor, and maturation during circadian are very limited in the fish.

Therefore, in the present study, we investigated the physiological rhythms of VAL-opsin and GTHs in goldfish by exposure to two kinds of LED light (red and green) and a white fluorescent bulb (control), and the differences between VAL-opsin and GTHs expression with and without GTH injection were examined. This study is the first report to focus on the relationship between VAL-opsin and reproduction of teleost fish during circadian exposed to various wavelengths.

2. Materials and methods

2.1. Experimental fish and conditions

For each experiment, immature goldfish (*Carassius auratus*) ($n = 400$, length, 6.1 ± 0.5 cm; mass, 12.5 ± 0.4 g) were purchased from a commercial aquarium (Choryang, Busan, Korea) and maintained in five 300-L circulation filter tanks prior to experiments in the laboratory. The five experimental conditions were reared in duplicate and with 40 fish per tank. The goldfish were reared in automatic temperature regulation systems (JS-WBP-170RP; Johnsam Co., Buchoen, Korea); the water temperature was maintained at 22 °C, and the fish were allowed to acclimate to the experimental conditions for 24 h.

In the present study, the experimental design for light condition was modified from the methods of Shin et al. [31]. The light control group was exposed to light from a white fluorescent bulb (27 W, wavelength range 350–650 nm); placed 50 cm above the water surface and the light intensity at the water surface was approximately 0.96 W/m^2. The experimental groups were exposed to red (peak at 630 nm) and green (530 nm) LEDs (Daesin LED Co. Kyunggi, Korea); placed 50 cm above the water surface. The light intensity at the water surface was approximately 0.96 W/m^2 (Fig. 1). The fish in the control and experimental groups were exposed to a 12-h light:12-h dark photoperiod (lights on at 07:00 h and lights off at 19:00 h). The fish were reared under these conditions with daily feeding of a commercial feed until the day prior to the sampling.

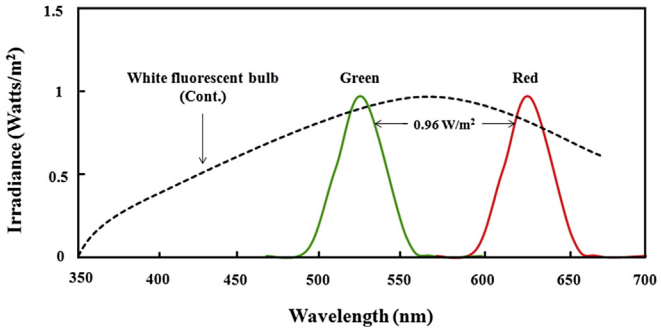

Fig. 1. Spectral profiles of the red (630 nm) and green (530 nm) light-emitting diodes (LEDs) used in this study. The dotted line shows the spectral profile of the white fluorescent bulb (Cont.). Reprinted from Shin et al. [28], with permission from *Comparative Biochemistry and Physiology Part A*. (For interpretation of the references to color in this figure caption, the reader is referred to the web version of this article.)

The spectral analysis of the lights was performed using a spectroradiometer (FieldSpec®, ASD, CO, USA). The fish were anesthetized with 200 mg/L 2-phenoxyethanol (Sigma, St. Louis, MO, USA) to minimize stress prior to blood collection. The fish were euthanized by spinal transection (first sampling at 11:00 h) at 4-h sampling intervals (ZT4, ZT8, ZT12, ZT16, ZT20, ZT24, ZT36, and ZT48) to collect the hypothalamus, pituitary, and blood under dim light using an attenuated white fluorescent bulb. Plasma samples were separated from blood sample by centrifugation (4 °C, 10,000 × *g*, 10 min) and stored at −80 °C until analysis. The tissue samples were removed from the fish, immediately frozen in liquid nitrogen, and stored at −80 °C until analysis.

2.2. GTH injection

To investigate the effects of GTH on VAL-opsin expression, the fish were anesthetized with 2-phenoxyethanol (Sigma, St. Louis, MO, USA) prior to injection. GTH (Human chorionic gonadotropin; WAKO, Osaka, Japan) was dissolved in 0.9% physiological saline, and each fish was injected with GTH (5 μg/g and 10 μg/g, body mass [BM]) at a volume of 10 μL/g BM. The sham group was injected with an equal volume of 0.9% physiological saline (10 μL/g BM). The control groups are same as the control of light experiment. Each tank (each experimental group) included 40 fish. Four hours after the injection, the retina and hypothalamus samples were removed from the fish at 4-h sampling intervals for 2 days (first sampling at 11:00 h).

2.3. In vitro culture of cells

For cultures, the hypothalamus tissue was quickly removed at 07:00 h (lights-on time) and placed in 3 mL of ice-cold dispersion buffer (pH 7.4, Dulbecco's phosphate-buffered saline; Gibco-BRL, Rockville, MD, USA). The isolated hypothalamus tissues were then transferred to 6 mL of fresh dispersion buffer containing 0.25% trypsin (Type II-S from porcine pancreas; Sigma). The connective tissues and other impurities were removed, and the hypothalamus tissues were chopped into small pieces with a pair of scissors. The hypothalamus cells and minced hypothalamus tissue were transferred to a flask and incubated for 10 min at room temperature with slow stirring. The mixture of dispersed hypothalamus cells and tissues was filtered, and the culture medium (Gibco-BRL; osmolality adjusted to match the goldfish plasma osmolarity of 353 mOs) was added. The cell suspension was centrifuged at 800 × *g* for 10 min, and the cells were then resuspended in fresh culture medium. The hypothalamus cells at a concentration of approximately