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ルリスズメダイにおける生殖活動の光制御機構に関する分子生物学的研究

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Abstract

Most vertebrates living in the temperate zone perceive seasonal changes in their habitat environments based on photoperiod and begin the preparation for future reproductive performance by activating the hypothalamus-pituitary-gonadal (HPG) axis. In mammals exhibiting photoperiodism, information in relation to light-dark cycle light is received by the retina, and transmitted to the master clock in the suprachiasmatic nucleus of the brain and expressed as the circulating levels of melatonin, which is an indoleamine hormone with an increase during scotophase and a decrease during photophase. On the other hand, how photoperiodic information is transduced as internal signals and how the HPG axis is stimulated by internal signals remain unclear in fish. In addressing reproductive mechanisms in relation to photoperiodic responses in fish inhabiting coral reef, this study aimed to clarify (1) the mechanism of transducing photoperiodic information in the brain, (2) the mechanism of activating the HPG axis, and (3) the involvement of melatonin in regulating the HPG axis. The sapphire devil *Chrysiptera cyanea* – a reef-associated damselfish that is widely distributed in the shallow waters of the West Pacific Ocean and has high commercial value as an ornamental fish – was used as an experimental fish. The following results were obtained using the sapphire devil.

To investigate the transducing mechanism of photoperiodic information, effects of acute light exposure during scotophase on ocular melatonin content was examined. Exposing fish to blue (short) wavelengths of light, but not red (long) and green (middle) wavelengths of light, suppressed ocular melatonin synthesis. In situ hybridization revealed that melanopsin, a candidate photoreceptor molecule in the retina, is involved in suppressing ocular melatonin production. When long-afterglow phosphorescent pigment (LumiNova) pellets were placed on the calvaria of fish, ovarian development was induced during the nonbreeding season. It is suggested that fish can perceive light (from long to middle wavelengths) by photoreceptor(s) and start gonadal development.

To investigate the mechanism of activating the HPG axis, reproduction-related genes – kisspeptin genes (*kiss1* and *kiss2*), G protein-coupled receptor 54 (GPR54) genes (*gpr54-1* and *gpr54-2*), gonadotropin releasing hormone (GnRH) genes (*gnrh1*, *gnrh2*, and *gnrh3*) and gonadotropin β subunits (FSH β and LH β) genes (*fsh β* and *lh β*) – were cloned from the brain and characterized. When seasonal changes in mRNA abundance of the genes in the brain was examined by real-time quantitative PCR, most genes (*kiss1*, *kiss2*, *gpr54-1*, *gpr54-2*, *gnrh1*,

lh β , and fsh β) increased towards the late vitellogenesis phase, suggesting that these genes are related to the ovarian development. Treatment of estradiol-17 β (E2) stimulated the mRNA expression of these genes in the brain of immature fish, suggesting endocrine controls of the positive feedback mechanism. When sexually mature fish were treated with melatonin during the spawning season, mRNA abundance of kiss1, gnrh1, and lh β decreased within 3 h after treatment. Chronic treatment of fish with melatonin resulted in a decrease in gonadosomatic index within 2 weeks as well as increases in atresia in vitellogenic oocytes and immature oocytes in ovaries. It is concluded that melatonin plays an important role in the reproductive mechanism in relation to the photoperiodism response and regulates the HPG axis (especially in Kiss1-GnRH1-LH cascade).