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Research Report

Photoperiodic changes in hypothalamic insulin receptor gene expression are regulated by gonadal testosterone

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ABSTRACT

In order to adapt to seasonal changes, animals exhibit robust changes in their reproductive status, body weight, and molt. However, the molecular mechanisms regulating such seasonal changes in physiology and behavior are not fully understood. Here, we report the photoperiodic regulation of the *insulin receptor* (*IR*) gene in the infundibular nucleus (anatomically homologous to the mammalian arcuate nucleus) of the Japanese quail. When the birds were transferred from short-day to long-day conditions, a significant increase in the level of *IR* mRNA was observed on the 10th long day, whereas that in testicular length was observed on the 5th long day. Castration abolished *IR* mRNA expression induced by long-day conditions, whereas the testosterone administration mimicked induction of *IR* mRNA expression induced by long-day conditions. These results suggested that the photoperiodic regulation of the *IR* mRNA in the infundibular nucleus is mediated by testosterone from the testes. It has been known that the central administration of insulin increases luteinizing hormone (LH) secretion, and neuron-specific disruption of *IR* gene causes impaired gonadal function due to the dysregulation of LH and increased food intake and body weight. Together with these results, the photoperiodic regulation of the *IR* mRNA in the hypothalamus may enhance the effect of long days in the seasonal response of reproduction and body weight changes.

1. Introduction

In order to adapt to seasonal changes in the environment, animals exhibit robust changes in their reproductive status, body weight, and molt for maximal survival. For this seasonal adaptation, the annual changes in photoperiod are used as the primary cue. In long-day breeders such as quail and hamsters, the transfer from short photoperiod to long photoperiod increases reproductive activity, food intake, and body weight (Boon et al., 2000; Hoffman, 1973; Wade and Bartness, 1984). In

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contrast, transfer from long photoperiod to short photoperiod reduces these parameters. The mediobasal hypothalamus (MBH) is considered to be the center controlling the photoperiodic response in birds and mammals (Follett et al., 1998; Malpaux et al., 1998; Maywood and Hastings, 1995; Yoshimura et al., 2003; Yoshimura, 2004). Studies conducted in our laboratory have revealed the critical role of thyroid hormone conversion within the MBH in the photoperiodic response of gonads (Nakao et al., 2006; Watanabe et al., 2004; Yasuo et al., 2005; Yoshimura et al., 2003). These studies showed that hypothalamic triiodothyronine (T_3) concentration is precisely regulated by the reciprocal expression of thyroid hormone-activating (*Dio2*) and -inactivating (*Dio3*) enzyme genes in the MBH; additionally, increased T_3 concentration in the MBH under long-day conditions induces testicular growth. Although these studies advanced our knowledge on the regulation of photoperiodism, the molecular mechanism underlying seasonal changes in various physiological features and behavior is not fully understood.

The Japanese quail is an excellent animal model for studying photoperiodism (Follett et al., 1998; Yoshimura et al., 2003). Therefore, in the present study, we performed differential subtractive hybridization analysis to search for novel photoperiod-responsive genes in quail MBH and identified photoperiodic regulation of *insulin receptor* (*IR*) gene in the infundibular nucleus, which is anatomically homologous to mammalian arcuate nucleus (ARC). Temporal expression analysis revealed that testicular growth precedes *IR* mRNA induction. Therefore, we examined the effect of castration and testosterone administration on *IR* mRNA expression.

2. Results

2.1. *Photoperiodic regulation of IR mRNA in the infundibular nucleus of the Japanese quail*

Differential analysis revealed a high expression of *IR* mRNA under long-day conditions and low expression under short-day conditions in the infundibular nucleus (Student's *t*-test, $P<0.01$) (Fig. 1). No hybridization signal was observed in sense control (data not shown). When birds were transferred from short- to long-day conditions, a significant increase in testicular length was detected on the 5th day (one-way ANOVA, $F(11,36)=29.686$, $P<0.0001$, Fisher's least significant difference (LSD) post hoc test, $P<0.05$), while a significant increase in *IR* mRNA expression was detected on the 10th day (one-way ANOVA, $F(18,55)=2.846$, $P=0.0015$, Fisher's least significant difference (LSD) post hoc test, $P<0.05$) (Fig. 2).

2.2. *Effect of castration and testosterone on IR expression*

Castration (CX) abolished the *IR* mRNA expression induced by long-day conditions (one-way ANOVA, $F(3,8)=7.188$, $P=0.0117$, Fisher's least significant difference (LSD) post hoc test, $P<0.01$) (Fig. 3A). It was found that *IR* mRNA expression was induced in the infundibular nucleus by the testosterone implant (*t*-test, $P<0.01$) (Fig. 3B); this implant mimicked long-day-induced plasma androgen concentration (*t*-test, $P<0.01$) (Fig. 3D), cloacal gland growth (*t*-test, $P<0.01$) (Fig. 3E) and *P450arom* mRNA expression in the nucleus preopticus medialis (POM) (*t*-test, $P<0.01$) (Fig. 3C) of the castrated quail.

3. Discussion

In the present study, we observed the photoperiodic regulation of *IR* mRNA in the infundibular nucleus of the Japanese quail. In addition, when the birds were transferred from short-day conditions to long-day conditions, a long day induction of *IR* mRNA was observed on the 10th long day. This relatively slow induction was in contrast with the rapid changes in *Dio2* and *Dio3* mRNAs (Yasuo et al., 2005). Because testicular growth induced by long-day conditions preceded *IR* mRNA induction, we examined the effects of castration and testosterone administration on *IR* mRNA expression. As expected, castration abolished *IR* mRNA induction under long-day conditions, and testosterone implantation within a physiological dose rescued *IR* mRNA induction under short-day conditions. These results clearly demonstrated that the *IR* mRNA expression induced by long-day conditions in the infundibular nucleus is mediated by the testosterone secreted from the testes. Recently, Tups et al. (2006) reported *IR* mRNA expression induced by long-day conditions in the ARC of Siberian hamsters, which was consistent with the findings of the present study. Although they examined the effect of food deprivation and body weight changes on *IR* mRNA expression, no statistically significant correlation was observed. It appears possible that testosterone may also drive photoperiodic *IR* mRNA induction in the ARC of hamsters.

The pancreatic hormone, insulin, and leptin primarily secreted by adipocytes are known to enter the brain from circulation and act in the central nervous system (CNS) to reduce energy intake and body weight (Niswender et al., 2004; Plum et al., 2005; Schwartz et al., 2000). The neurons in the ARC express insulin and leptin receptors and are considered to integrate peripheral signals to maintain energy homeostasis. It is reported that mice with neuron-specific disruption of the *IR* gene (NIRKO mice) show increased food intake and diet-

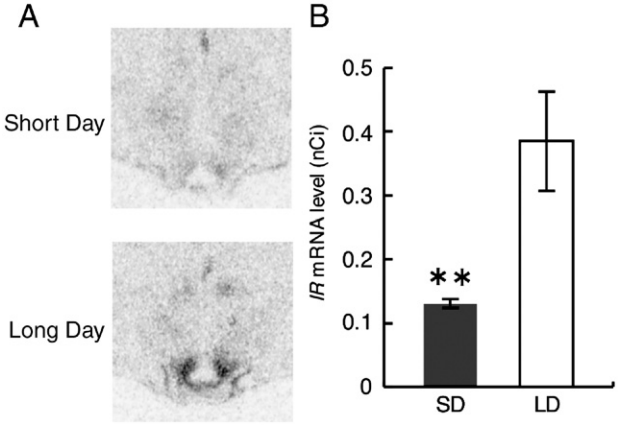

Fig. 1 – Photoperiodic regulation of the *insulin receptor* (*IR*) mRNA in the infundibular nucleus of the Japanese quail. (A) Representative autoradiograms of *IR* mRNA expression in the infundibular nucleus. (B) Expression of *IR* mRNA was high under long-day conditions and low under short-day conditions (Student's *t*-test, $P<0.01$, $n=4$).