Differential response of type 2 deiodinase gene expression to photoperiod between photoperiodic Fischer 344 and nonphotoperiodic Wistar rats

Shinobu Yasuo,1 Miwa Watanabe,1 Masayuki Iigo,2 Takahiro J. Nakamura,1 Tsuyoshi Watanabe,1 Tsyoshi Takagi,1 Hiroko Ono,1 Shizufumi Ebihara,1 and Takashi Yoshimura1,3

1Division of Biomodeling, Graduate School of Bioagricultural Sciences and 3Institute for Advanced Research, Nagoya University, Chikusa-ku, Nagoya; and 2Department of Applied Biological Chemistry, Nagoya Institute for Advanced Research, Graduate School of Bioagricultural Sciences and Institute for Advanced Research, Nagoya University, Chikusa-ku, Nagoya, Japan

Submitted 7 June 2006; accepted in final form 14 November 2006

FOR MANY SPECIES living in temperate zones, annual changes in photoperiod are the primary factor that regulates the timing of reproduction. The photoperiodic response allows animals to bear their offspring during the food-abundant season to maximize survival. On the other hand, laboratory rats have been bred for many generations to obtain maximal reproductive efficiency under constant temperature and light conditions with ad libitum food availability; this has resulted in the loss of reproductive responsiveness to photoperiod (27). Indeed, the Wistar and Sprague-Dawley inbred rat strains are generally regarded as nonseasonal breeders (23, 28, 36). Nevertheless, some experimental treatments such as neonatal androgen injection, chronic exposure to exogenous testosterone, and olfactory bulbectomy can induce a reproductive responsiveness to photoperiod in these rat strains (23, 28, 34, 36). Extreme lighting conditions, such as constant darkness or blinding, also inhibit gonadal development in Wistar rats (15). These reports indicate that neuroendocrine components that regulate photoperiodic response are still preserved in the Wistar and Sprague-Dawley strains. In contrast, the Fischer 344 (F344) inbred strain of rat exhibits robust photoresponsiveness, even under normal conditions, exhibiting slower gonadal growth on exposure to short days (11, 14). Gonadal development of F344 rats is also inhibited by blinding (17) or by melatonin injection (12). A recent study has also reported that the ACI, PVG, and BUF inbred rat strains are also photoperiod sensitive (6), suggesting that sensitivity to photoperiod varies across different rat strains. However, the underlying mechanism that regulates the variation in photoresponsiveness is not well understood.

In mammals, the photoperiodic response of gonads is regulated by a daily cycle of melatonin secretion from the pineal gland (1, 27). Melatonin synthesis and secretion occur during the night; therefore, in many species, the pattern of melatonin secretion is influenced by day length. For example, the melatonin secretion patterns of hamsters are greatly affected by the photoperiod, exhibiting a longer duration under short-day conditions and a shorter duration under long-day conditions (8, 16). These changes in duration of nocturnal melatonin secretion into the blood are generally accepted to be critical to melatonin’s effect on gonadal regulation (2, 5). Although the site at which melatonin acts to induce photoperiodic gonadal response has yet to be elucidated, lesion studies in Syrian hamsters have demonstrated the involvement of several brain regions, including the dorsomedial hypothalamus and the ventromedial hypothalamus of the mediobasal hypothalamus (MBH) (18, 21), the anterior hypothalamic nucleus (10), and the bed nucleus of the stria terminalis (26). Among these regions, the action of melatonin on the MBH seems to be conserved in various species. For example, melatonin microimplants placed in the premammillary hypothalamic area of the MBH produced a short-day effect on luteinizing hormone secretion in sheep, whereas implants located in other brain regions were ineffective (19, 20).

Long-day-induced type 2 iodothyronine deiodinase (Dio2) expression in the MBH is critical in the photoperiodic response of gonads in birds (42). Dio2 catalyzes the conversion of the prohormone thyroxine (T4) to bioactive triiodothyronine (T3) and controls the local thyroid hormone concentration (3, 42). Furthermore, in Japanese quail, T3 administration to the MBH mimics the long-day effect on gonadal regulation (38, 42). In mammals, the Dio2 expressed in the MBH also appears to be involved in the regulation of gonadal growth, since photope-
photoperiodic regulation of Dio2 expression is also observed in the MBH of the Djungarian hamster (37), the Syrian hamster (29), and the Saanen goat (39) and subcutaneous injection of T3 under short days mimics the long-day effect on testicular growth in Djungarian hamsters (7). Moreover, Dio2 expression is suppressed in both Djungarian and Syrian hamsters by daily injections of melatonin (29, 37), suggesting that in photoperiodic gonadal regulation Dio2 expression is involved in the pathway between the melatonin signal and the neuroendocrine-gonadal axis.

Therefore, in the present study, we assumed that the different photoresponsiveness among rat strains is determined by Dio2 expressed in the MBH. To test this hypothesis, we examined the effect of short and long days on Dio2 expression in the F344 and Wistar strains.

MATERIALS AND METHODS

Animals. All of the male F344 and Wistar-Imamichi 3-wk-old rats used in the present study were obtained from a local dealer and housed in light-tight boxes in which light cycles were provided. The boxes were placed in a room at a temperature of 24 ± 1°C. Since food restriction is known to enhance the suppressive effect of short days on reproductive maturation in F344 rats (13) and deer mice (22), a low-calorie diet (ZF for herbivorous animals; Oriental Yeast, Tokyo, Japan) was provided to both F344 and Wistar rats to maximize the photoperiodic response in this study. Food and water were available ad libitum and were replenished at least twice a week. The present study was approved by the Committee on Animal Experiments of the Graduate School of Bioagricultural Sciences, Nagoya University.

Effect of photoperiod on Dio2 expression. Rats were maintained under 8:16-h light-dark (8L16D) conditions before the experiment. At 7 wk of age, they were randomly divided into two groups; one group was transferred to the long-day conditions (16:8-h light-dark, 16L8D), while the other group was maintained under short-day conditions (8L16D). Rats were killed by decapitation at 9 wk of age, and the brains were collected for in situ hybridization. The brains were collected at midday (8 h after light onset under long-day conditions and 4 h after light onset under short-day conditions).

Daily melatonin injections. Rats were maintained under 14:10-h light-dark cycle (14L10D) throughout the experiment. The melatonin injection was administered according to Heideman et al. (12). At 5 wk of age, a daily subcutaneous injection of melatonin (100 µg of melatonin dissolved in 0.1 ml of 10% ethanol and 0.9% NaCl) or vehicle (0.1 ml of 10% ethanol/saline) was administered 1 h before the initiation of light offset. Injections were carried out for 3 wk followed by brain sampling.

In situ hybridization. In situ hybridization was carried out according to Yoshimura et al. (41). Antisense 45-mer oligonucleotide probes for Dio2 (5’T-tgtggaggaatgacgctagctgaggcaggtagaggagggagg-3’) were labeled with [32P]dATP (NEN Life Science Products, Boston, MA) using terminal deoxynucleotidyl transferase (GIBCO BRL, Frederick, MD). Coronal sections (20 µm thick) of the MBH were prepared with a cryostat. Hybridization was carried out overnight at 42°C. Two high-stringency posthybridization washes were performed at 55°C. The sections were air dried and apposed to Biomax-MR film (Kodak, Rochester, NY) for 2 wk. 14C standards (American Radiolabeled Chemicals, St. Louis, MO) were included in each cassette, and the relative optical density was measured with a computed image-analyzing system (MCID; Imaging Research, St. Catherines, ON, Canada) and converted into the radioactive value (nCi) with the 14C standard measurements.

RESULTS

Photoperiodic regulation of gonadal growth in F344 strain but not Wistar strain. We measured the testicular weights of F344 and Wistar strains maintained under short- or long-day conditions for 2 wk. The testicular weight of the Wistar strain did not differ between short and long days (Mann-Whitney U-test, P > 0.05), while that of the F344 strain differed significantly (Mann-Whitney U-test, P < 0.01) (Fig. 1). This result was consistent with previous reports that the Wistar strain is a reproductively nonphotoperiodic strain, while the F344 strain is sensitive to photoperiod (11, 12, 14, 17, 28).

Photoperiodic regulation of Dio2 mRNA in F344 strain but not Wistar strain. We examined Dio2 expression in the MBH of F344 and Wistar strains under short- and long-day conditions. In both strains, Dio2 mRNA was observed in the cellular zone overlying the tuberoinfundibular sulcus (TIS), which is localized in the lateral portion of the median eminence, and the ependymal cell layer lining the infralateral walls of the third ventricle (EC), as previously described in rats (9, 33) and the Djungarian hamster (37). When the expression levels of Dio2 in the TIS and EC were compared under short- and long-day conditions, a significant difference was observed in the TIS of the F344 strain (Mann-Whitney U-test, P < 0.05), while no difference was observed in the EC (Fig. 2). In contrast, photoperiodic treatment did not affect Dio2 expression in either regions of the Wistar strain (Mann-Whitney U-test, P > 0.05; Fig. 2).

Daily melatonin injections suppress Dio2 expression in both strains. Since daily melatonin injections during late afternoon suppress Dio2 expression in the MBH of Djungarian and Syrian hamsters (29, 37), we next examined the effect of daily melatonin injections on Dio2 expression in F344 and Wistar strains under long-day conditions. This experimental design is known to mimic the short-day effect on gonadal growth in the Syrian hamster (32), the Djungarian hamster (31), and the F344 rat (12). As a result, Dio2 expression in the TIS and EC was suppressed in both F344 (Mann-Whitney U-test, P < 0.01) and Wistar (Mann-Whitney U-test, P < 0.01) strains (Fig. 3).
DISCUSSION

The photoperiodic regulation of Dio2 in the MBH has been demonstrated to play a critical role in the photoperiodic regulation of gonads (42). In the case of long-day breeders such as the Japanese quail and Djungarian and Syrian hamsters, Dio2 expression in the MBH is higher under long-day conditions than under short-day conditions (29, 37, 40, 42). Similarly, the results of this study demonstrated higher Dio2 expression in the TIS of the photoperiod-sensitive F344 strain under long-day conditions; this was accompanied by an increase in testicular mass. In contrast, Dio2 expression and testicular mass of the Wistar strain were not affected by the photoperiod as recently reported (29). These results support the hypothesis that in rats, reproductive seasonality is also regulated by the photoperiodic regulation of Dio2 expressed in the MBH.

It is well established that patterns of nocturnal melatonin secretion from the pineal gland control the photoperiodic gonadal response (1, 27) and that melatonin acts on the melatonin binding site within the MBH (18, 21). In a previous study, we demonstrated (37) that the Dio2 mRNA expressed in the MBH of Djungarian hamsters is suppressed by daily melatonin injections administered during the late afternoon under long-day conditions. The suppression of Dio2 mRNA by melatonin is also reported in Syrian hamsters (29). Therefore, we assumed that the responsiveness of Dio2 to the melatonin signal might differ between the F344 and Wistar strains. Thus we next examined the effect of daily melatonin injections on the Dio2 expression in the MBH of both strains. Surprisingly, Dio2 expression was suppressed in both strains. Testicular volume was also suppressed in both strains, although a slight individual variation was observed in the Wistar rats (data not shown). Previous research has also demonstrated that daily melatonin injections in the late afternoon under long days or under a 12:12-h light-dark cycle inhibit testicular development in F344 rats (12) and young Wistar rats (30). These results suggest that both strains are capable of responding to melatonin. Although there is a possibility that changes in testosterone level affected the Dio2 mRNA levels as a secondary effect, this does not appear to be the case. Revel et al. (29) reported that castration under long days or implantation of testosterone under short days does not affect Dio2 mRNA levels in Syrian hamsters.

The similar responsiveness of Dio2 mRNA to melatonin injections in both strains suggests that strain differences of reproductive responsiveness to photoperiod would not be caused by the sensitivity of the reproductive axis to melatonin signals. This hypothesis would be distinct from the case of F344 and Sprague-Dawley rats, because the reproductive system and regulatory system for body mass of Sprague-Dawley rats are unresponsive to melatonin signals, even if melatonin patterns are identical in both strains (25). The differences between Wistar and Sprague-Dawley rats are also obvious in the fact that gonadal development of Wistar rats is inhibited by constant darkness or melatonin injections (15, 30), whereas that of Sprague-Dawley rats does not respond to these treatments (25). Instead, our data lead to the possibility that patterns of melatonin secretion under short- and long-day conditions may differ between Wistar and F344 strains. Indeed, our preliminary data obtained from samples collected every 4 h suggest that the amplitude of nocturnal melatonin peak in F344 rats was higher under short-day than long-day conditions, whereas no differences were seen in Wistar rats (data not shown). It is interesting to note that some reports emphasize not only the duration but also the amplitude of the nocturnal melatonin peak as an important seasonal parameter in gonadal regulation in many photoperiodic animals whose melatonin amplitude varies with the seasons (4, 24, 35). To test this hypothesis, it is important to examine the detailed melatonin profile in F344 and Wistar rats in the future.

ACKNOWLEDGMENTS

We thank the Nagoya University Radioisotope Center for the use of their facilities.
Evidence that melatonin acts in the premammillary hypothalamic area to control reproduction in the ewe: presence of binding sites and stimulation of lutinizing hormone secretion by in situ microimplant delivery. Endocrinology 139: 1508–1516, 1998.


REFERENCES


