Photoperiod Regulates Vitamin A and Wnt/\(\beta\)-Catenin Signaling in F344 Rats

Gisela Helfer,* Alexander W. Ross,* Laura Russell, Lynn M. Thomson, Kirsty D. Shearer, Timothy H. Goodman, Peter J. McCaffery, and Peter J. Morgan

The Rowett Institute of Nutrition and Health (G.H., A.W.R., L.R., L.M.T., P.J.Mo.), University of Aberdeen, Bucksburn, Aberdeen AB21 9SB, Scotland, United Kingdom; and Institute of Medical Sciences (K.D.S., T.H.G., P.J.Mc.), University of Aberdeen, Aberdeen AB25 2ZD, Scotland, United Kingdom

In seasonal mammals, growth, energy balance, and reproductive status are regulated by the neuroendocrine effects of photoperiod. Thyroid hormone (TH) is a key player in this response in a number of species. A neuroendocrine role for the nutritional factor vitamin A has not been considered, although its metabolic product retinoic acid (RA) regulates transcription via the same nuclear receptor family as TH. We hypothesized that vitamin A/RA plays a role in the neuroendocrine hypothalamus alongside TH signaling. Using a reporter assay to measure RA activity, we demonstrate that RA activity levels in the hypothalamus of photoperiod-sensitive F344 rats are reduced in short-day relative to long-day conditions. These lower RA activity levels can be explained by reduced expression of a whole network of RA signaling genes in the ependymal cells around the third ventricle and in the arcuate nucleus of the hypothalamus. These include genes required for uptake (\(Ttr\), \(Stra6\), and \(Crbp1\)), synthesis (\(Raldh1\)), receptor response (\(RAR\)), and ligand clearance (\(Crapb1\) and \(Cyp26B1\)). Using melatonin injections into long-day rats, we show that the probable trigger of the fall in RA is melatonin. Surprisingly we also found \(RPE65\) expression in the mammalian hypothalamus for the first time. Similar to RA signaling genes, members of the Wnt/\(\beta\)-catenin pathway and \(NMU\) and its receptor \(NMUR2\) are also under photoperiodic control. Our data provide strong evidence for a novel endocrine axis, involving the nutrient vitamin A regulated by photoperiod and melatonin and suggest a role for several new players in the photoperiodic neuroendocrine response. (*Endocrinology 153: 815–824, 2012*)

Photoperiod has profound effects on physiology, such as growth, energy balance, and reproduction, through changes in the neuroendocrine axis. In birds and mammals, control of seasonal reproductive physiology involves local regulation of thyroid hormone (TH) signaling within the hypothalamus. In the past decade, the significance of this TH signaling pathway has become evident, and the molecular and physiological mechanisms are now being elucidated. The active TH form \(T_3\) is synthesized from \(T_4\) by the activating deiodinase enzyme (DIO2) in the tanycytes surrounding the third ventricle of the hypothalamus (reviewed in Refs. 1 and 2). Recent work in Japanese quail and sheep has shown that TSH, derived from the pars tuberalis gland of the pituitary, mediates the photoperiodic drive to hypothalamic TH metabolism (3, 4).

A signaling factor regulating transcription via the same nuclear receptor binding partner, retinoid X receptor (RXR), as TH is retinoic acid (RA), the metabolic product of vitamin A. RA is best known for its effects during embryonic development (5). In the adult brain, RA signaling has been described in only a limited number of brain regions and functions, including the hippocampus where...
RA modulates neurogenesis, neuronal survival, and plasticity (6–9); the olfactory system where it regulates neuronal plasticity (10); regions involved in songbird vocal/auditory learning (11); the nucleus accumbens where it controls dopaminergic signaling (12); and in central nervous system remyelination (10, 13). Sites of RA synthesis in the brain are also limited to regions such as the meninges, cerebellum, and ventral tegmentum from where it can act in a paracrine fashion in the mature central nervous system (10). Recent data indicate that RA signaling also needs to be considered in the adult hypothalamus (14) and especially so in the photoperiodic response of seasonal animals (15, 16). Related to this, it is also interesting to note that the hypothalamus has been reported as a site of neurogenesis (17). In the hypothalamus of Siberian hamsters, the cellular binding protein transcripts, Crbp1 and Crabp2, are down-regulated by short photoperiod (short day [SD]), indicating seasonal control of cellular transport of retinol (15, 18). Likewise, the nuclear RA receptor (RAR) and RXRγ mRNA are reduced in the hypothalamus in SD compared with long days (LD). These changes are not present in pinealectomized hamsters held in SD, suggesting that the difference in expression is photoperiodically mediated (15). In addition, RA-synthesizing enzymes and the retinol transport protein stimulated by retinoic acid gene 6 homolog (STRA6) have been localized in the tanycytes lining the third ventricle of the mouse (16), a brain region that is involved in the regulation of seasonal physiology. More significantly, this is also the case in photoperiodically responsive F344 rats (16). Furthermore, using microarray analysis, RA signaling genes have been identified as one of three prominent gene sets that are differentially expressed in the hypothalamus of F344 rats held in SD and LD accompanied by changes in physiology such as body weight and food intake (19).

As a nutritional product, rather than as part of a hormonal system, RA has not been considered part of any neuroendocrine regulatory circuits in the hypothalamus. However, our studies indicate that RA signaling, in addition to TH-dependent pathways, may be involved in the neuroendocrine control of seasonal physiology. Here we provide a comprehensive study of RA signaling gene expression changes in the hypothalamus of F344 rats. We show that all major players of the RA pathway from uptake, through synthesis, catabolism, and receptor response, including the presence of the ligand are inhibited by short photoperiod. Melatonin, known to mediate the physiological effects of SD photoperiod in mammals (20), seems to be responsible for this decline. The temporal dynamics of photoinduction of RA-responsive genes relative to seasonal physiological responses have been considered. We also identified additional new players in the photoperiodic response of the hypothalamus, such as neuromedin U (NMU) and Wnt/β-catenin signaling, which may act upstream and downstream, respectively, of hypothalamic RA and/or TH signaling.

**Materials and Methods**

**Animals**

Male F344 rats aged 3–4 wk were obtained from Harlan Sprague Dawley Inc. (Indianapolis, IN). After acclimatization, rats were single housed in constant temperature (21 ± 2 °C). Food (CRM (P) Rat and Mouse Breeder and Grower, standard pelleted diet; Special Diet Services, Witham, Essex, UK) and water were provided ad libitum. The retinoid content of the diet was β-carotene, 1.28 mg/kg; retinol, 5.22 mg/kg; and vitamin A, 17,376.4 IU/kg. All animal procedures were approved by a local ethics committee and licensed under the Animals (Scientific Procedures) Act, 1986.

**Photoperiod experiment**

After approximately 1 wk of acclimatization in a 12 h light/d photoschedule, 48 male F344 rats aged 5–6 wk were divided into weight-matched groups of eight rats per group. Rats were then transferred to LD (16 h light/d) photoperiod or to SD (8 h light/d) photoperiod rooms. After the appropriate number of days in photoperiod, one group of rats from each photoperiod was anesthetized using isoflurane inhalation and killed by decapitation at zeitgeber time (ZT) 3. Brains were dissected and immediately frozen on dry ice and then stored at −80 °C. To determine the relative RA levels by reporter assay, eight male F344 rats aged 5–6 wk were divided into two weight-matched groups, one placed in LD and the other in SD for 28 d. Rats were anesthetized as above and decapitated. Brains were removed and hypothalamic and hippocampal tissue immediately dissected for the RA reporter assay described below.

**Daily melatonin injections**

Rats used in this study were also used for gene expression analysis reported in another recent publication and thus are fully described in Ross et al. (19). Briefly, male F344 rats were housed initially in LD (14 h light/d) photoperiod after arrival. Food intake and body weights were recorded daily and three times per week, respectively. Melatonin injections were administered according to Heideman et al. (21). For both the 3- and 14-d duration experiments, rats aged 5–6 wk were divided into three weight-matched groups of eight rats, with one LD group injected daily with melatonin (100 µg melatonin dissolved in 0.1 ml 10% ethanol and 90% physiological saline; delivered sc) and LD and SD (10 h light/d) photoperiod control groups injected daily with vehicle (0.1 ml 10% ethanolic saline). Injections were carried out 1 h before lights off (ZT13 for the LD groups and ZT9 for the SD group) and continued for 3 or 14 d at which time rats were anesthetized using isoflurane inhalation and decapitated during the mid-light phase (8 h after lights on for LD and 4 h after lights on under SD). Brains were immediately removed and frozen on dry ice and then stored at −80 °C.
Photoinduction experiment

As above, rats used in this study were the same as previously used and described (19). Male F344 rats were initially exposed to SD (10 h light/d) photoperiod. Food intake and body weights were recorded daily and three times per week, respectively. At 5–6 wk of age, rats were divided into weight-matched groups of eight rats. Two groups of eight rats stayed in SD, and two groups of eight rats were transferred to LD (14 h light/d) photoperiod. After 3 d, one SD and one LD group were killed; the remaining rats were killed after 14 d. Killing through decapitation after isoflurane inhalation took place during the mid-light phase as described above.

Hypothalamic gene expression

F344 rat hypothalamus coronal sections were cut and in situ hybridization was performed as previously described in detail (22). Riboprobe templates were prepared as described earlier for hybridization was performed as previously described in detail (23).

NMUR2

mouse 925-bp DNA template corresponding to 2507–3431 bp of the sequence (GenBank accession no. NM_013467) and cloned into pBluescript KS (Agilent Technologies). Sense riboprobes were synthesized from a 398-bp DNA template (GenBank accession no. NM_053562) amplified from Siberian hamster hypothalamic cDNA by PCR using forward primer 5′-ATGCGAGAGTAAAGGTGGCCAGC-3′ and reverse primer 5′-GGACCTCCCCTTTCAATCTCTT-3′ and cloned into Topo Zero Blunt PCR vector (Invitrogen, Paisley, UK). The plasmid was received as a gift from Dr. Hamada (Osaka University, Osaka, Japan). Except where stated otherwise, the following riboprobe templates were amplified from rat hypothalamic cDNA and cloned into the vectors indicated following the manufacturer’s instructions. 

Raldh1 riboprobes were prepared from a 398-bp DNA template corresponding to 833-1230 bp of the mouse Raldh1 sequence (GenBank accession no. NM_013467) and cloned into pBluescript KS (Agilent Technologies, UK Ltd., Edinburgh, UK). Crabp1 riboprobes were prepared from a 398-bp DNA template (GenBank accession no. JN031030), which was amplified from Siberian hamster hypothalamic cDNA by PCR using forward primer 5′-TATGTCCGGGAGTAAAGGTGGCCAGC-3′ and reverse primer 5′-CGACGGAGAATTTCGACGAG-3′ and cloned into pGEM6 (Promega, Southampton, UK). The plasmid was received as a gift from Dr. Hamada (Osaka University, Osaka, Japan). Except where stated otherwise, the following riboprobe templates were amplified from rat hypothalamic cDNA and cloned into the vectors indicated following the manufacturer’s instructions. 

Raldh1 riboprobes were prepared from a 398-bp DNA template corresponding to 833-1230 bp of the mouse Raldh1 sequence (GenBank accession no. NM_013467) and cloned into pBluescript KS (Agilent Technologies). Sense riboprobes were synthesized from a 398-bp DNA template corresponding to 833-1230 bp of the mouse Raldh1 sequence (GenBank accession no. NM_013467) and cloned into pBluescript KS (Agilent Technologies). Sense riboprobes were synthesized from a 398-bp DNA template corresponding to 833-1230 bp of the mouse Raldh1 sequence (GenBank accession no. NM_013467) and cloned into pBluescript KS (Agilent Technologies).

Statistical analysis

Data were analyzed by Student’s t test or one-way factorial ANOVA with Tukey’s honestly significant difference post hoc test as appropriate using SigmaPlot version 11.0 statistical software (Systat Software Inc., Chicago, IL). The results are presented as mean ± SE. P < 0.05 was considered statistically significant.

Results

A reduction in hypothalamic RA signaling is a SD photoperiodic response

Using Affymetrix microarrays, we have identified significant photoperiod-mediated changes in expression of genes involved in the RA signaling pathway within the hypothalamus of F344 rats (19). We have mapped the temporal and spatial expression of RA signaling genes involved in uptake, synthesis, response and metabolism of RA and their changes in response to LD photoperiod (16 h light/d) and SD photoperiod (8 h light/d) in F344 rats using in situ hybridization.

Vitamin A in the form of retinol circulates bound to retinol-binding protein (RBP) and transthretin (TTR) (25). Uptake into cells involves the membrane receptor for RBP, STRA6 (26). Both STRA6 and TTR mRNA are strongly expressed in the ependymal layer lining the third ventricle of the hypothalamus (Fig. 1, A and B), and the expression of STRA6 and TTR is decreased in short photoperiod relative to long photoperiod [one-way ANOVA, F(5,41) = 3.934, P = 0.001 for Ttr; and F(5,38) = 39.34, P < 0.001 for Stra6]. The magnitude of differential expression between long and short photoperiod increases with time after photoperiod switch (Fig. 1, A and B), and this parallels the changes in physiology, including body weight and food intake (19). The conversion of retinol into RA involves cellular RBP1 (CRBP1), which promotes uptake and presentation of retinol to synthetic enzymes. Retinol dehydrogenase catalyzes conversion of retinol to
then retinaldehyde dehydrogenase (RALDH) converts retinal into RA (26). Crpb1 and Raldb1 are expressed in the ependymal layer of the third ventricle in F344 rats (Fig. 1, C and D). Both Crpb1 and Raldb1 are significantly reduced in the F344 rat after 28 d in short photoperiod (t test, \( t_{16} = 18.15, P < 0.001 \), Fig. 1C for Crpb1; and \( t_{19} = 26.56, P < 0.001 \), Fig. 1D for Raldb1).

RA catabolism in the brain is mediated by CYP26B1 (27), and the activity of the catabolic enzymes is enhanced by CRABP1 (28, 29). Both genes are under direct positive feedback by RA (30, 31) to limit excess amounts of RA. These genes are expressed mutually in the ependymal layer, extending into the arcuate nucleus (ARC), particularly Crpb1, and they are down-regulated together in the hypothalamus under SD photoperiodic conditions (t test, \( t_{18} = 19.37, P < 0.001 \), Fig. 1E for Crbp1; and \( t_{13} = 10.56, P < 0.001 \), Fig. 1F for Cyp26B1).

The RA signal is transduced by the RAR, which activates transcription as a heterodimer associated with RXR, which itself is either unliganded or bound to 9-cis-RA (26). Using a RAR probe, which recognizes all isoforms of RAR (15), strong expression of RAR mRNA is present in the ARC, the ependymal layer of the third ventricle and the dorsomedial posterior ARC (dmpARC). Expression of the RAR markedly declines under SD conditions compared with LD (t test, \( t_{17} = 5.82, P < 0.001 \) in the ependymal region/ARC; and \( t_{17} = 7.72, P < 0.001 \) in the dmpARC, Fig. 2A). In contrast, although the pattern of expression was similar to RAR, levels of expression of RXR\( \gamma \) are stronger in SD than in LD in the ARC and ependymal layer (t test, \( t_{15} = -4.81, P < 0.001 \), Fig. 2B). In the dmpARC, a strong RXR\( \gamma \) signal is detectable, but this is unaffected by photoperiod (Fig. 2B).

Retinal pigment epithelium 65 (RPE65) is an all-trans:11-cis retinol isomerase of the visual cycle (32), which is mainly expressed in the retinal pigment epithelium. We found RPE65 expression in the dorsal part of the ependymal layer and the optic chiasm of F344 rats (Fig. 2C), but densitometry at 1, 3, and 14 d in photoperiod did not show a significant difference of RPE65 mRNA expression.

**Relative RA activity levels in the hypothalamus of F344 rats**

Although RAR\( \alpha \), RAR\( \beta \), and RAR\( \gamma \) are expressed throughout the mature central nervous system (33), RA synthesis and signaling occurs only in a very limited number of brain regions (6–10). Our findings that RA signaling genes are present and photoperiodically responsive in the hypothalamus, however, suggest that the hypothalamus might be an additional brain region in which RA synthesis occurs. We used a reporter assay to investigate whether the relative levels of RA activity in the hypothalamus of F344 rats are affected by photoperiod. Relative RA activity levels were examined in F344 rats that were acclimated in LD (16 h light/d) and SD (8 h light/d) for...
Consistent with the changes in RA signaling gene expression, we found higher relative levels of RA activity in the hypothalamus of rats on LD compared with SD (t test, t_{6} \approx \text{2.75}, P < 0.001, Fig. 3). By contrast, measurements of the relative levels of RA activity in the hippocampus from the same animals showed no differences between the two photoperiods (Fig. 3).

**Inhibition of RA signaling gene expression by melatonin**

Studies in hamsters and rats have shown that photoperiodic regulation of seasonal physiological responses, including changes in body weight, requires an intact pineal gland that synthesizes and releases melatonin and that SD responses can be mimicked in LD by daily injection of melatonin (34, 35). Likewise, an intact pineal gland is required to support photoperiod-regulated changes in Crbp1, Crabp2, RAR, and RXR in the hamster hypothalamus (15). This implicates melatonin in the regulation of hypothalamic RA signaling. We therefore administered melatonin to F344 rats housed in LD for 3 or 14 consecutive days to test whether this was sufficient to inhibit Stra6, Raldh1, and Cyp26B1 expression levels to those observed in SD rats. F344 rats were injected with melatonin or vehicle 1 h before lights off, corresponding to ZT13 and ZT9 under LD (14 h light/d) and SD (10 h light/d) conditions, respectively. 

Raldh1 transcript, encoding the enzyme that synthesizes RA, was rapidly reduced just 3 d after melatonin treatment of LD rats (one-way ANOVA, F(2,21) = 3.49, P < 0.001) and after 14 d expression was nearly absent in both SD control and LD melatonin-injected rats (F(2,19) = 104.82, P < 0.001, Fig. 4A). Stra6 and Cyp26B1 mRNA levels were unaffected after 3 d of melatonin treatment to LD rats (Fig. 4, B and C), but 14 d of melatonin treatment was sufficient to reduce Stra6 mRNA expression by about 50% (F(2,21) = 7.94, P = 0.003, Fig. 4B) and Cyp26B1 mRNA expression by 75% (F(2,19) = 8.70, P = 0.002, Fig. 4C). Thus, control of hypothalamic RA synthesis is under rapid control of melatonin, whereas other components of the RA signaling pathways such as Stra6 and Cyp26B1 are regulated less rapidly or secondarily.

**Responsiveness of RA signaling to photoinduction**

Because short photoperiod and melatonin have inhibitory effects on expression of RA signaling genes, it was important to determine whether this was reversible by photoinduction. Transfer of F344 rats equilibrated on SD (10 h light/d) to LD (14 h light/d) initiated an increase of the RA signaling genes Raldh1, Stra6, and Cyp26B1 within 3 d. Raldh1 mRNA expression is almost absent in
the ependymal layer of SD rats, and after transition to LD it markedly increases after 3 and 14 d [one-way ANOVA, $F(3,27) = 28.88, P < 0.001$, Fig. 4D]. *Str6* mRNA expression in the ependymal layer of LD rats increases by nearly 70% relative to SD rats, 3 d after transition. This increase continues for 14 d, by which time *Str6* mRNA levels are more than doubled [$F(3,28) = 20.93, P < 0.001$, Fig. 4E]. *Cyp26B1* mRNA also increases in LD after the transition [$F(3,28) = 5.61, P = 0.004$, Fig. 4F], but the increase is not as pronounced as for *Str6* and *Raldh1*.

These rapid changes in *Str6*, *Raldh1* and *Cyp26B1* gene expression occur clearly in advance of overt changes in physiology. Body weights and food intakes are significantly greater in LD rats after 11 d and 6 d, respectively, compared with SD rats (19). Hence, it is conceivable that these genes are involved in the regulation of energy balance.

**Wnt/β-catenin signaling**

A wide range of differentially expressed genes were identified on the microarray, including those involved in the Wnt/β-catenin signaling (19). We have selected two examples from the Wnt/β-catenin pathway that were differentially expressed on the microarray and mapped their expression in response to photoperiod. *Dickkopf3* (DKK3) belongs to a group of secreted glycoproteins that inhibit the Wnt/β-catenin pathway (36). Dkk3 mRNA levels were strongly influenced by photoperiod with a significant increase seen as early as 3 d in LD compared with SD [$F(5,41) = 35.22, P < 0.001$, Fig. 5A]. Another inhibitor of the Wnt/β-catenin signaling is the secreted frizzled-related protein 2 (sFRP2). *sFrp2* mRNA expression was higher in LD compared with SD after 28 d in photoperiod ($t_{14} = 22.45, P < 0.001$, Fig. 5B) suggesting that Wnt/β-catenin signaling is inhibited in LD and activated in SD in the hypothalamus of F344 rats. Both Dkk3 and sFrp2 genes are expressed in the ependymal layer and ARC of the hypothalamus, similar to RA signaling genes.
Photoperiodic control of NMU and NMU receptor 2 (NMUR2)

NMU is a hypothalamic neuropeptide that acts through the hypothalamic G protein-coupled receptor NMUR2. Although a profound role of NMU in the control of food intake and energy balance has been suggested (37), photoperiod responsiveness in seasonal animals has so far not been studied. We found pronounced expression of NMU mRNA in the pars tuberalis of the pituitary of F344 rats. The expression of NMU significantly increases as early as 14 d in LD compared with SD [one-way ANOVA, F(5,39) = 14.89, P < 0.001, Fig. 6A]. Likewise, the NMUR2, which is expressed in the ependymal layer and the ARC, is increased in long photoperiod relative to short photoperiod (t test, t19 = 14.89, P < 0.001, Fig. 6B), demonstrating photoperiodic responsiveness of NMU and its receptor NMUR2 in the relevant hypothalamic areas in F344 rats.

Discussion

Recent data have indicated that some RA signaling genes may have a role in photoperiodic responses in the hypothalamus that accompany physiological changes (15, 16). Results from this study considerably extend these preliminary findings by demonstrating that a whole network of genes involved in RA uptake, synthesis, catabolism, and receptor response, expressed in the hypothalamic ependymal cells and adjacent ARC, are inhibited by short photoperiod and by melatonin in F344 rats. Importantly, these changes are associated with marked reduction in hypothalamic relative RA levels. The suppression of RA signaling gene expression by short photoperiod is also reversible through photoinduction. Given that it is known that RA depletion inhibits food intake and growth in rats (38, 39), and it is proposed that this may involve a hypothalamic mechanism (39), then it seems plausible to suggest that photoperiod-induced changes in RA signaling genes and RA levels may contribute to the reduced food intakes and body weights observed in SD-exposed F344 rats.

The key locus for vitamin A/RA signaling within the hypothalamus are the ependymal cells surrounding the third ventricle, because it is these cells that express all the genes involved in the uptake, signaling, and metabolism of vitamin A/RA. Vitamin A circulates in the blood in the form of retinol, an alcohol, bound to a RBP/TTR complex (25). Uptake of retinol by cells is mediated by the specific membrane-bound receptor for RBP, STRA6 (26). Thus, the differential expression of Stra6 observed in different photoperiods, suggests that under LD a higher rate of retinol uptake occurs into ependymal cells. The higher expression of Ttr in LD relative to SD is also consistent with this. Although, because Ttr also acts as a carrier for the TH hormone T4 (26), it is possible that ependymal cell expression of Ttr, as opposed to plasma levels, may be more important to TH than to retinol uptake. Previously, Ttr expression in the hypothalamus of Siberian hamsters has been reported, although the site of expression was not clearly defined and photoperiod responsiveness appeared limited to the photorefractory state (40).

Consistent with a higher level of retinol uptake under LD, two genes involved in the synthesis of RA, Crbp1 and Raldh1, were shown to be expressed at higher levels in LD relative to SD. Previously we have reported photoperiodic regulation of Crbp1 expression in the ependymal cells of the Siberian hamster (15), but photoperiod regulation of the essential RA-synthesizing gene, Raldh1, has not been reported before. These data in combination with measurements of relative RA activity levels using a novel and sensitive reporter assay, strongly indicate that the hypothal-

FIG. 6. NMU and NMU2 expression in the hypothalamus of F344 rats determined by in situ hybridization. A, NMU mRNA expression in the pars tuberalis is significantly higher in LD (16 h light/d) compared with SD (8 h light/d) after 14 and 28 d in photoperiod (P < 0.01; ***, P < 0.001). B, NMUR2 mRNA expression is significantly higher in LD (16 h light/d) compared with SD (8 h light/d) after 28 d in photoperiod (P < 0.001). Representative autoradiographic images are shown. Scale bar, 1.0 mm. Data represent mean ± SE from eight animals per group. **, P < 0.01; ***, P < 0.001.
Catabolism of RA is also strongly regulated by photoperiod, because expression of two genes, Cyp26B1 and Crabp1, involved in RA catabolism is strongly inhibited in SD relative to LD conditions. This pattern of expression suggests a lower rate of RA catabolism under SD and, in combination with a lower rate of RA synthesis, suggests a lower rate of flux of RA under SD. Consistent with a reduced vitamin A uptake, synthesis, and metabolism under SD, a reduced receptor response is also predicted because the expression of the RAR decline under SD compared with LD conditions. This is similar to the pattern of RAR expression previously observed in the Siberian hamster (15). In contrast to the RAR, RXRγ levels were up-regulated in SD in the ARC region of F344 rats. The up-regulation of RXRγ is different from the hamster, where RXRγ is down-regulated in SD conditions similar to RAR (15, 42). In addition to RAR, RXR is also a binding partner for a number of other nuclear receptors, including TH receptors (43). Therefore, it is possible that the up-regulation of RXRγ in the ARC of F344 rats may be necessary for its involvement in the regulation of other pathways.

Thus, overall, from uptake through synthesis to receptor response, all of the major players of the RA signaling pathway are altered to decrease the potency of this transcriptional signal in the hypothalamus under SD. This triggers a state of relative RA depletion in the hypothalamus that, given previous evidence, is likely to influence downstream responses such as growth and food intake (38, 39).

A surprising result from our microarray was the expression of the RPE65 gene in the hypothalamus (19). RPE65 is important to RA metabolism because it participates in the conversion of all-trans retinol to 11-cis retinal during phototransduction in photoreceptor cells (44), but its expression outside the retina in mammals has not been shown before and is therefore noteworthy. We found RPE65 expression in the dorsal part of the ependymal layer and the optic chiasm of F344 rats, although this pattern of expression was unaffected by photoperiod. A homolog of RPE65 has been identified in the brain of zebrafish (45), but extraretinal photoreceptors located in the hypothalamus are not unexpected in nonmammalian vertebrates (46). The mammalian circadian clock, however, receives photic information exclusively from the retina to entrain central circadian oscillators located in the suprachiasmatic nucleus of the hypothalamus. It is conceivable therefore that the presence of RPE65 in the hypothalamus of rats reflects that the RA signaling system located in the ependymal cells of the hypothalamus and adjacent ARC is a residue of a primitive light-sensitive endogenous clock.

In this study, we found that in addition to TH signaling and RA signaling genes, potential downstream RA target genes from the Wnt/β-catenin signaling pathway are among those showing the greatest differences in expression in the hypothalamus of F344 rats in response to altered photoperiod. Wnt/β-catenin signaling is known to have important roles during development, but more recently, roles in the adult nervous system, such as synaptic maintenance and function, as well as in adult neurogenesis have been identified (47). We investigated two inhibitors of the Wnt pathway, Dkk3 and sFrp2, that are under photoperiodic control inhibiting Wnt signaling in LD in the hypothalamus. Our data confirm the photoperiodic responsiveness of these Wnt signaling genes, but the functional roles of these changes remain to be determined. Like Wnt/β-catenin signaling, RA has been implicated in adult neurogenesis within the hippocampus (10). RA treatment modulates various Wnt proteins and receptors during neuronal differentiation (48) and in the dentate gyrus of the adult mouse, where adult neurogenesis has been confirmed, members of the Wnt signaling family appear to be downstream components of the RA response (8). To date, RA signaling has not been implicated with adult neurogenesis in the hypothalamus, but it is feasible that RA is required in the seasonal plasticity of the mature hypothalamus (17) with Wnt signaling as a potential downstream effector.

The role of melatonin in the photoperiodic regulation of RA signaling seems likely to involve the pars tuberalis, rather than direct action on the hypothalamus. This is because melatonin receptors are known to be abundantly expressed in the pars tuberalis of the rat but have only limited distribution in the hypothalamus (49). Furthermore, the regulation of TH signaling in the hypothalamus has been shown to be mediated by TSH, and the pars tuberalis is an important site of TSH biosynthesis, which is under photoperiodic regulation (3, 19, 50). From this, a retrograde neuroendocrine pathway between the pars tuberalis and the tanycytes of the hypothalamus has been proposed where TSH acts as a hormonal intermediate (3, 50).

NMU is another potential hormonal intermediate, which conveys photoperiodic information between the pars tuberalis and the ependymal cells of the hypothalamus. NMU was identified as strongly photoperiodically regulated from our microarray studies of the hypothalamic extracts from F344 rats (19). Like TSHβ and choricionic glycoprotein alpha (the common glycoprotein alpha subunit of glycoprotein hormones), NMU is
expressed in the pars tuberalis of the pituitary, and its receptor NMUR2, like the TSH receptor, is expressed in the ependymal layer and ARC, and both are also strongly regulated by photoperiod.

To date, NMU has been reported as a hypothalamic neuropeptide that has been associated with the control of energy balance (37). For example, intracerebroventricular administration of NMU reduces food intake, induces stress-related behavior, and stimulates the hypothalamic-pituitary axis (37, 51–53). These effects are thought to be mediated by the hypothalamic NMUR2 (51), which is expressed in the same regions as the TH, RA, and Wnt signaling genes. Despite the known central effects of NMU (37, 51–53), it is unclear how NMU may be involved in the neuroendocrine control of seasonal body weight and food intake regulation. This is especially so, because contrary to expectation, the highest levels of NMU are associated with the highest levels of food intake under LD. It is intriguing to hypothesize that NMU might be involved in the photoperiodic drive to regulate the hypothalamic TH and/or RA metabolism, in a similar way to TSH (3, 4), which may be distinct from any direct role in the control of food intake. It remains to be established whether such an inverse neuroendocrine axis is part of the mechanism regulating retinoid signaling in the hypothalamus and whether either TSH or NMU is involved in the control of the thyroid and/or retinoid signaling pathways.

In conclusion, we provide strong evidence for a novel endocrine axis in which the nutrient vitamin A is involved in the regulation of the neuroendocrine hypothalamius with Wnt signaling as a potential downstream pathway and NMU as a potential upstream regulator. RA seems to act as a key intermediary between changes in photoperiod and the resulting physiological changes such as growth, energy balance, and reproduction. It is evident that there is coordinated signaling of TH and RA in response to photoperiod because the temporal profile of changes in RA signaling genes and TH signaling-related genes Dio2, Dio3, and TSH-β (19) in the hypothalamius of F344 rats are similar after the switch from SD to LD. It remains to be determined how these pathways are interlinked. In addition, our data further emphasize the importance of the ependymal layer of the third ventricle as a key interface between peripheral signals and the neuroendocrine hypothalamus.

Acknowledgments

Address all correspondence and requests for reprints to: Peter John Morgan, The Rowett Institute of Nutrition and Health, University of Aberdeen, Bucksburn, Aberdeen AB21 9SB, United Kingdom. E-mail: p.morgan@abdn.ac.uk.

We acknowledge the Scottish Government Rural and Environment Scientific Analysis and Services and Biotechnology and Biological Sciences Research Council Grant BB/G014272/1 for financial support of this work.

Disclosure Summary: The authors have nothing to disclose.

References

17. Kokoeva MV, Yin H, Flier JS 2005 Neurogenesis in the hypothal-
20. Goldman BD 2001 Mammalian photoperiodic system: formal properties and neuroendocrine mechanisms of photoperiodic time measurement. J Biol Rhythms 16:283–301
42. Kliwer SA, Umesono K, Mangelsdorf DJ, Evans RM 1992 Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D3 signalling. Nature 355:446–449