Photoperiodic Regulation of Histamine H3 Receptor and VGF Messenger Ribonucleic Acid in the Arcuate Nucleus of the Siberian Hamster


Molecular Endocrinology Group (P.B., A.W.R., A.B., P.A.L., J.G.M., K.M.M., C.U., P.J.M.), Rowett Research Institute and Aberdeen Centre for Energy Regulation and Obesity, Aberdeen AB21 9SB, Scotland, United Kingdom; Department of Biology (T.S., J.K., P.P.), Åbo Akademi University, Artillerigatan 6 20520, Åbo, Finland; Neuroscience Center (P.P.), Institute of Biomedicine/Anatomy, University of Helsinki, 00290 Helsinki, Finland; and School of Biomedical Sciences (S.S., F.J.E.), University of Nottingham Medical School, Nottingham NG7 2UH, United Kingdom

To survive winter the Siberian hamster has evolved profound physiological and behavioral adaptations, including a molt to winter pelage, regression of the reproductive axis, onset of daily torpor and increased capacity for thermogenesis. However, one of the most striking adaptations is the catabolism of intraabdominal and sc fat reserves contributing to the loss of up to 40% of body weight. These physiological and behavioral adaptations are photoperiodically driven, yet neither the site(s) in the brain nor the molecular mechanism(s) involved in the regulation of these profound adaptations is known. Here we report a dynamic regulation of gene expression in a dorsal region of the medial posterior area of the arcuate nucleus (dmpARC) of the Siberian and Syrian hamster brain in response to altered photoperiod. We show mRNA for the histamine H3 receptor is down-regulated and VGF is up-regulated in the dmpARC in hamsters switched from long- to short-day photoperiod. These data provide further evidence to support the view that the dmpARC is a major site to relay photoperiodic changes and as a site for the long-term regulation of seasonal physiology and behavior. (Endocrinology 146: 1930–1939, 2005)

SEASONAL MAMMALS UNDERGO robust changes in physiology and behavior enabling them to survive annual cycles of climatic and environmental change. The Siberian hamster anticipates seasonally reduced food availability during winter by a reduction of food intake even in conditions of ad libitum supply of food (1, 2). This results in the loss of up to 40% of body mass, mostly in the form of adipose tissue. The increasing duration of darkness as winter approaches also initiates reproductive quiescence (3). It is clear that the altered nocturnal duration of melatonin provides the neuroendocrine cue, reflecting photoperiod, to regulate seasonal physiology and behavior (4, 5). Receptor mapping studies have identified the location of the melatonin receptors within the brain and at peripheral sites (6). These studies in combination with lesioning and melanotin implant studies have revealed the importance of the hypothalamus to photoperiodically regulated changes in reproduction and energy homeostasis (7, 8). However, the underlying neural basis of voluntary decreased food intake or reproductive quiescence in these anticipatory mechanisms is not understood. The seasonal changes in prolactin that are important to the pelage response appear to be mediated independently of the hypothalamus and involve the pituitary pars tuberalis (7, 9).

Recently progress toward understanding the mechanisms that underpin photoperiodic responses has been made. It has been shown that light-induced type II deiodinase (DIO2) expression, responsible for the conversion of T4 to T3 is involved in the photoperiodically controlled gonadal response of the Japanese quail (10). It has also been reported that the expression of DIO2 within the ependymal layer of Djungarian hamsters is suppressed in short days (SDs) relative to animals under long days (LDs) (11), suggesting a potentially generic involvement of this enzyme and thyroid hormones in the photoperiod response of seasonal species.

Studies from our laboratory have revealed profound changes in the expression of several genes involved in retinoic acid receptor (RAR) and rexinoid receptor signaling in response to change from LD to SD in Siberian hamsters (12). This study showed that expression of cellular retinoic acid binding protein II (CRABP II), cellular retinol binding protein I (CRBP I), RAR, and retinoid X receptor (RXR)γ mRNAs are all suppressed in a region of the hypothalamus initially described as the dorsal tuberomammillary nucleus (DTMN) of...
SD- relative to LD-acclimatized hamsters (12). CRBP1 was also expressed in the ependymal layer of the third ventricle of the Siberian hamster and was shown to be under photoperiodic control (12). In combination these studies have identified new components and structures involved in photoperiodic signaling within the hypothalamus.

The seasonal body weight response in the Siberian hamster provides a useful index of the overall photoperiodic response because it is a noninvasive and repeatable measure of physiological status. From this it has been determined that the Siberian hamster is able to track its temporal position within a photoperiodic cycle and use this information to regulate energy balance accordingly (13).

A considerable body of evidence suggests that many of the hypothalamic factors known for their role in the acute or homeostatic control of energy balance, such as neuropeptide Y, proopiomelanocortin, cocaine- and amphetamine-regulated transcript, Orexin, melanin-concentrating hormone, and agouti gene-related peptide, are unlikely to have primary roles in seasonal body weight regulation. Furthermore, male Siberian hamsters bearing experimentally induced lesions of the majority of the arcuate nucleus (ARC), in which a number of the hypothalamic factors are produced or act, still show SD-induced decreases in food intake and body weight (14). Similarly the cycle in gonadal status is not simply reflected through overt changes in either the expression of GnRH gene or peptide (15). Thus, distinct factors must be involved in the mechanism that provides the interface between the photoperiodic (melatonin) input and the energy balance and reproductive axes.

Two factors are considered in this study. The first is histamine, which may be a central component of a hibernation mechanism (16), an alternative seasonal strategy used by some mammalian species to minimize energy expenditure over winter. Histamine has also been implicated in the regulation of both food intake (17–19) and reproduction (20) in nonseasonal species.

The second is the polypeptide VGF (nonacronymic) that is abundantly expressed in the brain. It is also found in the pituitary, adrenal, gut, and pancreatic tissues (21–23). Im-

tered transcript, Orexin, melanin-concentrating hormone, and agouti gene-related peptide, are unlikely to have primary roles in seasonal body weight regulation. Furthermore, male Siberian hamsters bearing experimentally induced lesions of the majority of the arcuate nucleus (ARC), in which a number of the hypothalamic factors are produced or act, still show SD-induced decreases in food intake and body weight (14). Similarly the cycle in gonadal status is not simply reflected through overt changes in either the expression of GnRH gene or peptide (15). Thus, distinct factors must be involved in the mechanism that provides the interface between the photoperiodic (melatonin) input and the energy balance and reproductive axes.

Two factors are considered in this study. The first is histamine, which may be a central component of a hibernation mechanism (16), an alternative seasonal strategy used by some mammalian species to minimize energy expenditure over winter. Histamine has also been implicated in the regulation of both food intake (17–19) and reproduction (20) in nonseasonal species.

The second is the polypeptide VGF (nonacronymic) that is abundantly expressed in the brain. It is also found in the pituitary, adrenal, gut, and pancreatic tissues (21–23). Importantly it has been implicated in the regulation of both energy balance and reproduction (24–26).

This study reveals profound changes in gene expression related to both histamine signaling and VGF in response to short photoperiods, which occur in the dorsal region of the medial posterior area of the ARC. These data add further weight to the importance of this area of the ARC as a center for photoperiodic signaling within the hypothalamus of Siberian and Syrian hamsters.

**Materials and Methods**

**Animals**

Experiments were conducted on male Siberian and Syrian hamsters, individually housed with *ad libitum* access to food and water. LD photoperiod hamsters were housed in a 16-h light, 8-h dark cycle; SD photoperiod hamsters were housed in an 8-h light, 16-h dark cycle. Hamsters were caged 3 h after lights on (Zeitgeber time, ZT3) except to assess gene expression over a 24-h period when animals were caged every 4 h using red light illumination for those animals to be culled during the dark phase. All brains were immediately dissected, frozen on dry ice, and then stored at −80°C until required.

**RNA isolation and cDNA synthesis**

Blocks of hypothalamic tissue were obtained from frozen tissue by manual dissection, cutting from approximately bregma −2.7 to −0.34 based on the mouse brain atlas (27). Poly A+ RNA was isolated using the oligotex kit (Qiagen, Crawley, West Sussex, UK).

For cDNA synthesis, 1 μg DNase-treated polyA+ RNA was randomly primed and reverse transcribed with Superscript II reverse transcriptase (Invitrogen, Paisley, UK) at 42°C. One tenth of the cDNA synthesis reaction was used for PCR with the following primers based on mouse, rat, or human sequences present in the GenBank database: 1) histidine decarboxylase (GenBank accession no. X97437), forward primer, 5′-CCCA CAT GCA CCG CTA CTA; reverse primer, 5′-GCC TCT GTC CCT GTC TTC TCT A; the PCR amplification temperatures were 94°C for 30 sec, 50°C for 30 sec, and 68°C for 1.5 min for 40 cycles; 2) histamine H1 receptor (GenBank accession no. AF388052), forward primer, 5′-CCG CCC CTC TGC TGC TTT TGT; reverse primer, 5′-TCT TGC CAC CTT TGG AAT CTT; and 3) histamine H3 receptor (H3R) (GenBank accession no. NM_133849), forward primer, 5′-GGA GCA CAG CCG CAC CCC GTC CTC GAC; and reverse primer, 5′-MTC CTC CTC CTC CTC GCT TGT GGC TCA CAG CAC; the PCR amplification temperatures were 94°C for 45 sec, 60°C for 45 sec, and 72°C for 2 min 20 sec for 30 cycles using Pfu Turbo polymerase (Stratagene, La Jolla, CA). The histamine H3R and VGF DNA fragments were cloned from hamster hypothalamic cDNA, whereas histamine H1 receptor and histidine decarboxylase were cloned from mouse hypothalamic cDNA. The resulting PCR fragments were cloned into either pGEM-T (Promega, Southampton, UK) or PCRscript (Invitrogen) and verified by sequencing.

**Cloning full-length H3R splice variants**

A Siberian hamster hypothalamic cDNA library was constructed from poly A+ RNA primed with an Xho-oligo dT primer in the vector pZAP II, with the aid of the Uni-ZAP XR library construction kit (Stratagene). The library was screened with the H3R PCR fragment generated with the above primers. Full-length clones for two splice variants of the H3R were isolated and sequenced. These sequences have been deposited in GenBank (Accession no. AY855070 and AY855071).

**cAMP assay**

Human embryonic kidney (HEK)293 cells were transfected with the splice variants of the H3R using the lipofectamine plus transfection reagent (Invitrogen), in 90-mm petri dishes. Twenty-four hours after transfection, the cells were trypsinized and counted. Approximately 2 × 10⁶ cells were seeded into wells of 24-well plates and remained in culture for the next 16–24 h. Each plate in turn was washed twice in ice-cold serum-free media. Treatment solutions were made by the dilution of drugs in complete media containing 0.1% dimethyl sulfoxide, and cooled on ice. The concentration of drugs used in this study were H3R agonist IMETIT (IME) 10⁻⁸ M; β₂-adrenergic receptor agonist isoproterenol (ISO) 10⁻⁸ M; and the H3R antagonist thiopiemamide (THI) 10⁻⁶ M. Treatment media were added to the cells. The plate was then incubated at 37°C for 30 min in a waterbath. Media were removed from the cells for assay of CAMP content. Ten microliters of media was subsequently used for a RIA of CAMP as described previously (28).
**Immunohistochemistry**

LD and SD hamsters were anesthetized with sodium pentobarbital. Perfusion was carried out with 4% N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide (Sigma, Dorset, UK), and brain storage was as described (16).

Twenty-micrometer cryostat sections were collected on gelatin-coated slides and air dried for 30–60 min. The sections were washed with PBS containing 0.25% Triton X-100 (PBS-T) and incubated with the antihistamine rabbit antiserum HA19C (30) diluted 1:20,000 in PBS-T with 2% normal goat serum. After the incubation (overnight at 4 °C), the sections were washed twice for 10 min with PBS-T and then incubated with Alexa 488 goat antirabbit IgG (Molecular Probes, Leiden, The Netherlands) diluted 1:500 in PBS-T, for 1 h at room temperature. The samples were washed with PBS and overslipped with PBS and glycerol (1:1). Fluorescence was detected with a TCS-SP confocal laser scanning microscope system (Leica, Quebec, Canada) equipped with an Ar-Kr laser (Omnichrome, Melles Griot, Carlsbad, CA). Fluorophores were sequentially excited with the wavelength peak at 488 nm. Image stacks were acquired and processed into maximum projection images with TCS NT/SP Scanware (version 1.6.587, Leica) software.

**HPLC**

LD and SD Siberian hamster brains 3 h after lights on (ZT3) were collected and frozen. With the aid of the mouse brain atlas, they were dissected into 10 regions. Each piece was weighed before determining histamine content.

Histamine content in the tissue was determined as described (16) by automated HPLC-fluorometric method (31).

**Statistical analysis**

Statistical tests employed in this study were t tests for the comparison of LD vs. SD responses to changes in gene expression in the dorsal region of the medial posterior area of the ARC (dmpARC), two-way ANOVA for comparison of photoperiod on histamine content in individual brain regions, and a one-way ANOVA with a post hoc Tukey test for the effect of H3R ligands on cAMP synthesis. The data were analyzed using the SigmaStat statistical software package (Jandel, San Ramon, CA).

**Results**

**Photoperiodic responses in the Siberian hamster**

Body weight and testis weight data for the Siberian hamsters have been documented previously (12). The Siberian hamsters used in these experiments demonstrated a 32% reduction in body weight in SD, compared with LD.

**Expression of HDC in the Siberian hamster brain**

Strong expression of HDC mRNA was detected by in situ hybridization in widely distributed neurons of the tuberomamillary nucleus as described for the ground squirrel, mouse, and rat (16, 32, 33) (Fig. 1A). The intensity of the autoradiographic signal indicates a high level of expression. No changes in regional expression or in mRNA abundance were observed in hamsters sampled on either long or short photoperiods at ZT3 (data not shown).

**Expression of the histamine H1 receptor mRNA**

Widespread distribution of the H1 receptor mRNA was detected in the brain of the male Siberian hamster. In the hypothalamus, high levels of expression were found in the ventromedial hypothalamic nucleus and the ARC (Fig. 1B) but no changes in distribution or abundance of H1 receptor mRNA in either the hypothalamus or other regions of the brain between animals housed in long or short photoperiod culled at ZT3 (data not shown).

**Expression of the H3R**

PCR amplification of histamine H3R sequences from hamster hypothalamic cDNA resulted in two DNA fragments of approximately 500 and 350 bp. Sequencing of these DNA fragments revealed they were alternative splice variants of the histamine H3R, with alternative splicing deleting 144 bp (48 amino acids) of the third intracellular loop, the region of G protein-coupled receptors that effects coupling with signaling G proteins (34, 35).

In situ hybridization of a H3R probe (recognizing all splice variants) on brain sections from male Siberian hamsters housed in LD reveals widespread distribution of the H3R mRNA throughout the brain, as recorded elsewhere for the rat (36). Within the hypothalamus, expression of H3R mRNA was particularly strong in the ARC, ventromedial nucleus, and the neurons of the tuberomamillary nucleus that express the enzyme HDC. The latter location is consistent with the known function of the histamine H3R acting as an autoreceptor to regulate the synthesis and release of histamine. However, the area with the highest levels of the H3R mRNA expression is the dmpARC (Fig. 2A). Analysis of H3R mRNA expression sampled at circadian time ZT3 revealed a reduction of expression to one third in the dmpARC in Siberian hamsters after 12 wk in SD, compared with those maintained in LD (Fig. 2, A and B). Emulsion-coated slides show that expression of the...
H3R mRNA decreases to background levels in SD animals in this region (Figs. 3, A and C, and 4, A and B). This region of high H3R expression (dmpARC) shows a distinct toluidine blue staining of densely packed cell bodies (Fig. 2C). Based on the mouse brain atlas and the high density of H3R mRNA expression we observed, we initially assigned this area of the brain as the DTMN (12). However, subsequent in situ hybridization experiments for HDC, a marker of histaminergic tuberomamillary neurons reveals no HDC expression in the dmpARC and only scattered cells lateral or dorsal to this region (Fig. 3, A and C). Colocalization studies with H3R show expression of the H3R mRNA on HDC-expressing neurons but no obvious difference between LD and SD for the expression of H3R on these HDC-positive cells (Fig. 3, A–D). Furthermore, immunohistochemical studies did not reveal histamine in cell bodies within the photoperiodic region (see below). Therefore, we tentatively refer to this region as the dmpARC.

Recently we reported that the expression of genes from the retinoid-signaling pathway, including RXRγ, RAR, CRBP1, and CRABP II, are also photoperiodically regulated in the dmpARC of Siberian hamsters (12). Dual in situ hybridization demonstrates H3R and RXRγ colocalize to the same cells in the dmpARC (Fig. 4).

Photoperiod effect on H3R expression is mediated by the pineal gland

Male Siberian hamsters held in LD were either pinealectomized or sham operated before transfer to SD for 12 wk. After they were killed, the animals were analyzed for H3R expression by in situ hybridization. H3R expression in the dmpARC was significantly higher in pinealectomized hamsters, compared with sham-operated controls (Fig. 5). This suggests the long duration of melatonin in SD is responsible for the decreased expression of the H3R. Although melatonin is regulated by the circadian clock (37), no circadian variation in H3R expression was observed over a 24-h period (data not shown) in either LD or SD.

Testosterone has been shown to regulate gene transcription in the brain (38, 39). Thus, because testicular regression in SD results in reduced testosterone, we assessed whether the reduction of testosterone in SD could affect expression of H3R in the dmpARC by using testosterone implants in SD hamsters. However, testosterone implants used to restore LD levels of testosterone in SD-exposed hamsters (12) did not...
alter the expression of H3R mRNA in the dmpARC (data not shown).

The H3R expression in the dmpARC of the Syrian hamster

Syrian hamsters were housed in long or short photoperiod for 14 wk. At the end of this period, the mean body weight and epididymal fat pad weights were significantly different between the two groups of animals (mean body weights (± se) excluding testis: LD, 115.0 ± 2.5 g, SD, 107.2 ± 1.4 g, n = 10, P = 0.014; fat pad weights, LD, 2.06 ± 0.14 g, SD, 1.22 ± 0.07 g, n = 10, P < 0.001. H3R gene expression in the dmpARC, determined by in situ hybridization, decreased in SD by 40% (P < 0.01) (Fig. 6)

VGF expression changes with photoperiod

Analysis of VGF gene expression by in situ hybridization showed that this gene had a widespread pattern of expression in the brain of the Siberian hamster, with a distribution similar to that reported for the rat and mouse (24, 40). This includes the ARC, paraventricular nucleus, lateral hypothalamic area, and high levels of expression in the suprachiasmatic nucleus (SCN). Comparison of VGF gene expression

Histamine innervation and histamine levels

Histamine measurements were made in 10 different regions of brain from Siberian hamsters kept in LD or SD. High levels were found in the hypothalamus, preoptic area, and thalamic region. There was no significant effect of photoperiod on the histamine content in the various regions analyzed (Fig. 8A).

Immunohistochemistry revealed the presence of histamine in fibers throughout the hamster brain as well as the cell bodies of the tuberomammillary nucleus. There is an absence of staining for histamine within cell bodies of the dmpARC, suggesting that histamine is not synthesized in the dmpARC (Fig. 8, B and C). Consistent with this, in situ hybridization did not detect HDC mRNA in the dmpARC of Siberian hamsters (Fig. 4, A and B).

Immunohistochemistry revealed no difference in the density of histaminergic fiber innervation of the dmpARC between LD and SD animals (Fig. 8, B and C).

Signal transduction by the histamine H3R

For the analysis of the ability of the Siberian hamster H3R splice variants to couple to the cAMP signal transduction pathway, the full-length Siberian hamster histamine H3R splice variants were transiently expressed in HEK293 cells together with the human β2-adrenergic receptor. The H3R agonist IME inhibited ISO-stimulated cAMP accumulation. This was partially blocked by the H3R antagonist THI for both splice variants of the histamine receptor (Fig. 9).
Discussion

The results of this study strengthen the view that the dmpARC is an important integrating center mediating photoperiodic responses in Siberian and Syrian hamsters. In addition to the changes in gene expression encoding components of the retinoic acid signaling pathway (12), we now demonstrate that photoperiod also regulates the expression of mRNAs encoding the H3R and VGF mRNAs within the dmpARC. Several aspects of these new observations are of interest. First, the directions of change are opposing: as H3R mRNA expression decreases, VGF mRNA expression increases. Second, these two genes have highly significant functions: the H3R as a signaling molecule has implications for cellular responses to histamine in LD, whereas VGF as a secretory protein has implications for the secretory output of the dmpARC. Third, given the ARC is a key area of the brain for maintaining normal homeostatic mechanisms of energy balance, the dmpARC is strategically placed to interact with those regions of the ARC involved in the acute responses to energy demand and in the long-term maintenance of seasonal body weight.

Photoperiodic regulation of histamine H3Rs

The potential importance of the histaminergic system for seasonal physiology in the Siberian hamster is supported by the key role it has been shown to play in the annual cycle of hibernation of the ground squirrel (16, 41–43). In this species, histamine synthesis and H3R regulation are likely to be key components in the mechanism of hibernation. In the Siberian hamster, however, a change in histamine synthesis is not likely to contribute to a change in seasonal physiology because neither HDC expression (data not shown) nor gross histamine content in 10 different regions of the brain varied between LD and SD. However, in the Siberian hamster, regulation of the H3R may play a role in seasonal physiology.

By in situ hybridization, the expression of histamine H3R mRNA was dramatically regulated by photoperiod, although this effect was extremely site specific, occurring in the dmpARC. This area of hybridization was initially described as the DTMN (12), based on two criteria, comparison with tuberomamillary nuclei assignment in the mouse brain atlas (27) and the known expression of H3Rs on histamine synthesizing neurons of the tuberomamillary complex.

However, HDC expression is a characteristic feature of histaminergic tuberomamillary neurons. Because the HDC-expressing neurons were located in a more dorsolateral position than the photoperiodically responsive H3R-expressing neurons in the Siberian hamster brain, it seems unlikely that
the latter constitute part of the DTMN. Based on the neuroanatomical subdivisions described in the mouse brain atlas (27), it would seem that the region of photoperiodically responsive H3R expression should be more appropriately considered a subregion of the ARC, a region tentatively described as the dmpARC. Further characterization of this region is required, which may provide evidence for a new subdivision of the ARC.

The dmpARC is well innervated by histamine-containing fibers, but there is no apparent difference in immunohistochemical staining for histamine between LD and SD in this brain region. These data lead to the hypothesis that altered expression of the H3R is the principal means to regulate histaminergic signaling input to the dmpARC.

Within the tuberomamillary nucleus, H3Rs are known to act as both autoreceptors, regulating histamine synthesis and release of histamine (44), and as heteroreceptors, controlling the release of other neurotransmitters and peptides such as serotonin, dopamine, γ-aminobutyric acid, noradrenaline, and acetylcholine (reviewed in Ref. 45). Because the dmpARC does not contain histamine-synthesizing neurons, the H3Rs are likely to serve as heteroreceptors in this region, suggesting that the function of the H3Rs in the dmpARC is to regulate the release of another bioactive compound.

**Photoperiodic regulation of VGF expression**

The increase in VGF mRNA expression in SD suggests that the dmpARC is involved in the synthesis and release of a neuropeptide or transmitter, which may relay SD signals from the dmpARC to other areas of the hypothalamus and brain. Whether VGF is a primary or secondary secretory product involved in such neural communication is at present unclear. Uncertainty exists over its role as either a structural component of the secretory granule or the precursor to bioactive peptides (26). In support of the latter, recent evidence demonstrates that C-terminal cleavage products of VGF increase synaptic charge in cultured hippocampal neurons (46). A similar action of VGF produced by the dmpARC would suggest that VGF might activate dmpARC efferents facilitating the SD signal beyond this nucleus. It is interesting to note that in regions of the ARC outside the dmpARC, a decrease in VGF mRNA expression in response to SD was observed. This may indicate decreased synaptic activity from these regions of the ARC in SD. The relationship and connections between the dmpARC and the other regions of the ARC are not known at this stage.

An obvious question is whether there is a functional relationship between VGF and the H3R. One plausible link is through the second messenger cAMP. Both splice variants of the Siberian hamster H3Rs reported here couple to the inhibition of cAMP synthesis. The loss of H3Rs would remove both histamine signaling via the H3R and remove any potential for constitutive activity of H3R to suppress the cAMP generation (47). This in turn would allow cAMP to rise or allow a stimulatory input to the dmpARC to increase cAMP and drive VGF expression, which is known to be positively regulated by cAMP (48, 49). Thus, in principle a functional connection appears plausible, but it remains to be proven.

**The relationship of VGF and H3Rs to seasonal physiology**

Siberian hamsters undergo many physiological and behavioral changes with altered photoperiod, including loss of body fat and reproductive quiescence. VGF has been implicated in both body weight and reproduction through targeted deletion of the VGF gene in mice (24, 25). Similarly, the H3R has been implicated in the regulation of energy homeostasis (but not reproduction) through targeted gene deletion in mice and pharmacological studies (50–52). However, the direction of change in expression of both the H3R and VGF with altered photoperiod found in this study is contrary to that anticipated from the gene knockout studies. In transgenic H3R null mice, an increase in body weight was observed, and VGF null mice are lean (24, 25, 50); therefore, after a decrease of H3Rs or an increase in VGF in the dmpARC, an increase in body weight might be expected. However, the effect on body weight of a gene deletion is the consequence of global ablation of the targeted gene both during the course of development and in the adult. Because the H3R and VGF are expressed at many sites, it is not possible to extrapolate the findings of these gene knockout studies to the effects seen at only a restricted site within the adult brain. However, our findings are consistent with recent pharmacological data demonstrating the H3 antagonist A-331440 has significant antiobesity effects in mice (52).
**H3R and VGF expression in the Syrian hamster**

In terms of testicular regression, Siberian and Syrian hamsters exhibited similar responses to 14 wk exposure to short photoperiod. By contrast, there were marked differences in body weight loss between the two species, in which Syrian hamsters lost approximately 6.8% body mass and Siberian hamsters 32% body mass after 14 wk in short photoperiod. Interestingly epididymal fat pad mass decreased by 41% in Syrian hamsters over the same period, and therefore, measurement of adipose tissue mass may be a more appropriate measure of the metabolic effects of photoperiod than gross body mass in the Syrian hamster. Therefore, on the basis of current information, it is not possible to link the changes in either H3R or VGF expression specifically to changes in either the reproductive or metabolic axes in these two species.

**Is the dmpARC a major site for the relay of photoperiod?**

In combination with previous findings (12), the data from this study support the view that the dmpARC is a key site involved in integrating photoperiodic responses. Because the responses are also seen in the Syrian hamster, the gene expression changes described herein may be related to the general decoding of the photoperiod rather than being related to specific physiological behaviors. The demonstration that in the dmpARC the H3R is expressed in the same cells as those expressing RXRγ emphasizes coordinated regulation of these transcripts in response to altered photoperiod. The significantly higher level of both the H3R and RXRγ mRNAs (12) in the hamsters pinealectomized before transfer to short photoperiod strongly implicates melatonin in mediating the response (53), although we have not formally ruled out the possibility of another pineal product mediating this effect. However, because melatonin receptors are not expressed on cells of the dmpARC (Ref. 54 and our unpublished observations), the seasonal effect of melatonin must be mediated at an upstream site. This site could be the SCN because SD melatonin infusions to Syrian hamsters bearing SCN lesions do not show SD physiological responses (reviewed in Ref. 55).

In a previous study by Ebling et al. (14), male Siberian hamsters with an 80% lesion of the ARC, induced chemically by treatment with monosodium glutamate (MSG) during the neonatal period, were still responsive to short photoperiod showing both a decrease in body weight and voluntary food intake. These data implied that the photoperiodic responses of the hamster were independent of the ARC. Thus, a central question in this study was whether the dmpARC was spared from MSG lesioning. Figure 10 shows the Cresyl violet-stained sections from this previous study depicting both the rostral (Fig. 10, A and C) and caudal (Fig. 10, B and D) regions of the ARC in vehicle- and MSG-treated hamsters. MSG treatment caused an 80% reduction in cell number of the rostral ARC, compared with vehicle-treated hamsters (14) (Figure 10, A and C), whereas no apparent difference is seen in the more caudal region of the ARC (Fig. 10, B and D). Importantly, the region of the dmpARC remained intact, consistent with our hypothesis for a role in mediating photoperiod regulation of the body weight and reproductive responses.

**Acknowledgments**

The β2-adrenergic receptor was a kind gift of Dr. Ralf Jockers (Institut Cochin, Paris, France). We thank Pauline Young and Donna Henderson for the DNA sequencing.

Received November 5, 2004. Accepted December 16, 2004.
References

tional regulation of the neurospecific, neurotrophin-inducible vgf gene. Mol Cell Biol 17:1244–1253

Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.