Photoperiod regulates arcuate nucleus POMC, AGRP, and leptin receptor mRNA in Siberian hamster hypothalamus

JULIAN G. MERCER,1 KIM M. MOAR,1 ALEXANDER W. ROSS,1 NIGEL HOZZARD,2 AND PETER J. MORGAN1

1Molecular Neuroendocrinology Unit and 2Molecular Physiology Group, Aberdeen Centre for Energy Regulation and Obesity, Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, United Kingdom

Mercer, Julian G., Kim M. Moar, Alexander W. Ross, Nigel Hoggard, and Peter J. Morgan. Photoperiod regulates arcuate nucleus POMC, AGRP, and leptin receptor mRNA in Siberian hamster hypothalamus. Am. J. Physiol. Regulatory Integrative Comp. Physiol. 278: R271–R281, 2000.—Siberian hamsters decreased body weight by 30% during 18 wk in short day (SD) vs. long day (LD) controls. Subsequent imposed food deprivation (FD; 24 h) caused a further 10% decrease. In the hypothalamic arcuate nucleus (ARC), SDs reduced proopiomelanocortin (POMC) gene expression and agouti-related protein (AGRP) mRNA was elevated, changes that summate to reduced catabolic drive through the melanocortin receptors. There was no effect of photoperiod or food deprivation on neuropeptide Y (NPY), melanin concentrating hormone, orexin, or corticotropin-releasing factor mRNAs. Superimposed FD increased AGRP gene expression and caused a localized elevation of NPY mRNA in the ARC. Both adipose tissue leptin and ARC leptin receptor (OB-Rb) mRNAs were downregulated in SDs, whereas FD increased OB-Rb gene expression. Thus OB-Rb mRNA is differentially regulated by acute and chronic changes in plasma leptin in this species. In a separate experiment in LDs, AGRP gene expression was increased by 24 or 48 h FD, whereas POMC mRNA was downregulated in the caudal ARC. AGRP and NPY mRNAs were extensively coexpressed in the ARC, and their differential regulation by photoperiod and FD is suggestive of transcript-specific regulation at the level of individual neurons.

Phodopus; agouti-related protein; proopiomelanocortin; orexins; melanin-concentrating hormone

The regulation of body weight, and thus the maintenance of an appropriate balance between energy intake and energy expenditure, involves interactions between an array of central and peripheral signaling systems focused on critical integratory centers in the hypothalamus. These include the hypothalamic arcuate (ARC) and dorsomedial nuclei (DMH), the lateral hypothalamus (LHA), and, pivotally, the paraventricular hypothalamic nucleus (PVH). Much of our knowledge of these regulatory systems is based on rat and mouse studies that have examined either responses to imposed energetic manipulations such as food restriction or deprivation or models of genetic obesity. In contrast, there are relatively few data assessing the roles and interactions of different components of the signaling array under conditions of dynamic physiologically programmed body weight regulation. Such models may provide insight into the longer-term regulation of body weight in the normal animal. In this context, the seasonal model of body weight regulation provided by the Siberian hamster (Phodopus sungorus) has several compelling features and represents a fascinating model in which to examine these processes. This species exhibits a number of adaptive responses to transfer from a long photoperiod (long day; LD) to a short photoperiod (short day; SD); animals display large amplitude, reversible changes in body weight that are induced by manipulation of photoperiod alone.

The cloning of the leptin gene in 1994 (45) has been the catalyst for increased activity in the field of energy homeostasis. In addition to sites of action throughout the body, in which it may affect metabolism and energetics, leptin targets the central nervous system (5), providing prompt negative feedback to brain centers involved in the regulation of energy balance (13). The leptin receptor exists as a number of splice variants (6, 20, 42), and the receptor gene is expressed in key hypothalamic regulatory centers including those already mentioned (26). Recently, several new candidate hypothalamic neuropeptide and receptor systems have been implicated in the regulation of food intake and body weight (13, 44). Some of these signaling systems, such as the orexins (36) and agouti-related protein (AGRP) (33), were previously unknown. In other cases, peptides with well-established activity in other physiological systems such as melanin-concentrating hormone (MCH) (34) or proopiomelanocortin (POMC) products have also been demonstrated to affect energy homeostasis. The activity of many of these new and established neuropeptide systems can be regulated by leptin, with extensive coexpression evidence at the mRNA and protein levels. Thus, for example, leptin receptor mRNA is coexpressed with neuropeptide Y (NPY) and POMC mRNA in neurons of the ARC (7, 25), and immunohistochemistry also reveals numerous potential interactions (14). The ARC-PVH projection is best characterized as the critical axis for the orexigenic peptide NPY (17). The ARC is also the predominant site of expression of POMC and AGRP mRNAs (11), and the
PVH is a key site of melanocortin (MC) 4-receptor expression (32). The recent characterization of the MCH (34) and orexin (36) orexigenic systems in the LHA, an area that also expresses leptin receptor mRNA (26), has rekindled interest in a part of the hypothalamus that has long been considered to be important in energy balance.

Our earlier studies of the endogenous NPY and corticotrophin-releasing factor (CRF) neuropeptide systems after photoperiodic and energetic manipulation in the closely related Djungarian hamster (P. s. campbelli) examined these neuropeptides in the context of seasonal appetite and body weight regulation (28, 29). The orexigenic peptide NPY was upregulated by food deprivation (FD), but there was no difference in NPY gene expression or peptide levels between LD hamsters and animals with established weight change after 10 or 20 wk in SDs (28, 29). The magnitude of the response of NPY gene expression to FD was highly dependent on the photoperiodic history of the animal (29). The Siberian hamster is sensitive to exogenous NPY, increasing food intake after intracerebroventricular injection (4); there was no evidence of sensitivity changes in different photoperiods.

The hypothesis underlying the present study is that changes in leptin signaling or sensitivity and/or the activity of anabolic or catabolic hypothalamic neuropeptide systems drive the physiological changes in body weight in the Siberian hamster induced by photoperiod manipulation. Our examination of these systems coincident with seasonal weight change is complemented by characterization of short-term compensatory responses to negative energy balance. We quantified gene expression for leptin in adipose tissues and leptin receptor in the ARC, a key neural site at which the leptin signal is integrated into neuroendocrine pathways (7, 25, 37, 38). We also examined levels of mRNA for the orexigenic peptides NPY, AGRP, MCH, and orexins and the anorexigenic ligands encoded by the CRF and POMC genes. We have examined LD control hamsters and SD animals close to their body weight nadir and superimposed on each group a 24-h FD period. We also assessed the effects of more prolonged FD in LD hamsters.

MATERIALS AND METHODS

Animals. Male Siberian hamsters were obtained from Wrights of Essex (Chelmsford, UK) and were individually housed in LD (16:8-h light-dark cycle) conditions at 22°C. Food (Labsure pelleted diet; Special Diet Services, Witham, Essex, UK) and water were available ad libitum unless specified to the contrary. Two experiments were performed. In experiment 1, hamsters were divided into two groups of 11 matched for body weight, one of which was transferred to SD (8:16-h light-dark cycle) conditions, but with other environmental conditions unaltered. Eighteen weeks after transfer to SDs, five animals from each of the SD and LD groups were deprived of food for 24 h. All animals were then killed by cervical dislocation in the middle of the light phase. Blood was collected in lithium heparin tubes for assay of insulin and cortisol as described previously (28). We were unable to measure leptin in hamster plasma using an ELISA assay based on mouse or human antibody (15). Brains were frozen on dry ice and stored at −70°C. Retinoperitoneal (RWAT), epididymal (EWAT), and inguinal (IWAT) white adipose tissue depots and interscapular brown adipose tissue (IBAT) were dissected free from other tissues, weighed, and snap frozen in liquid nitrogen. In experiment 2, adult male hamsters, maintained in LDs throughout, were divided into three groups matched for body weight. Six animals were fed ad libitum, whereas two groups of five hamsters were deprived of food for 24 or 48 h. All animals were then killed, and brain, blood plasma, and samples of IWAT and EWAT were frozen for analysis.

Northern blotting of leptin mRNA from adipose tissue. Total RNA was extracted from white or brown adipose tissue samples (300–500 mg), fractionated on a denaturing agarose gel, and blotted onto Genescreen membrane (Biotechnology Systems, NEN) as described previously (29). The leptin probe was prepared with alpha-[32P]dCTP by random priming of a cloned cDNA from the Djungarian hamster using the High Prime DNA labeling system according to the manufacturer's instructions (Boehringer Mannheim). The base sequences of leptin cDNAs cloned from Djungarian and Siberian hamsters were identical (unpublished data). Membranes were hybridized for 1 h at 65°C in QuikHyb (Stratagene), washed twice for 15 min in 0.2× SSC-0.1% SDS at room temperature and once for 30 min in 0.2× SSC-0.1% SDS at 60°C. Autoradiographic images were analyzed using Image-Pro Plus densitometry software (Media Cybernetics). Messenger RNA values were corrected for differences in gel loading by stripping and reprobing membranes with a human G3PDH probe (Clontech). Final results were expressed as the ratio of integrated intensities of leptin and G3PDH mRNAs and normalized to the ad libitum-fed LD control group.

Hypothalamic gene expression. Messenger RNA levels for the leptin receptor and a number of appetite- or body weight-related neuropeptides were quantified by in situ hybridization in 20-µm coronal sections using techniques described in detail elsewhere (28, 29). Riboprobes complementary to fragments of the common extracellular sequence of the leptin receptor (OB-R) and the intracellular domain specific to the long splice variant of the receptor (OB-Rb) were generated from Djungarian hamster brain cDNAs cloned by RT-PCR using primer pairs and methods described previously (24, 26). NPY and CRF probes were generated from rat cDNAs generously provided by Drs. Sabol and Mayo, respectively. An MCH cDNA (305 bp) was cloned from rat hypothalamus using the primers 5'-ACGGCATTTTACTTTCGGC-3' and 5'-CTGAACTTGAATCTTCGCT-3' (GenBank M29712). PCR conditions were as described previously (26).

AGRP and POMC cDNA fragments were cloned from Siberian hamster hypothalamic cDNA with 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min, then finally one cycle at 72°C for 5 min. An orexin (orexin A and B) cDNA was cloned from rat hypothalamus using the same PCR conditions. All three DNA fragments were ligated into pGEM-T Easy, transformed into JM 109 cells and sequenced. The 229-bp fragment of hamster AGRP was amplified using the primers 5'-GTGTTCCCAGAGTTCCCAGGTC-3' and 5'-GCATCTCCATTTCCAAGCTGG-3' (GenBank K00648). The fragment cloned from the hamster was 85% identical to the mouse AGRP. This translated into nine amino acid differences clustered nearer the N-terminus. A POMC cDNA fragment of 344 bp was amplified using the primers 5'-GGGCAAGCGTGTTCCCAGAGTTCCCAGGTC-3' and 5'-GCATTCTGGCAGTGTTGTTTATG-3' (GenBank U89484). The fragment was 92% identical to mouse AGRP at the cDNA level. This translated into nine amino acid differences clustered nearer the N-terminus. A POMC cDNA fragment of 344 bp was amplified using the primers 5'-GGGCAAGCGTGTTCCCAGAGTTCCCAGGTC-3' and 5'-GCATTCTGGCAGTGTTGTTTATG-3' (GenBank U89484). The fragment was 92% identical to mouse AGRP at the cDNA level. This translated into nine amino acid differences clustered nearer the N-terminus.
generated by RT-PCR using the primers 5'-CAGCCTCTGC-CCGACTCTGT-3' and 5'-CCGGCAGGAGAGGGAAAGT-3' (Genbank AF041241).

Hypothalamic sections were collected onto sets of eight slides, with adjacent sections on consecutively numbered slides. This permitted a number of mRNAs of interest to be localized and quantified in brain sections that were representative of different hypothalamic regions. Briefly, slides were fixed, acetylated, and hybridized overnight at 58°C using [35S]-labeled cRNA probes (1–2 × 10⁷ counts·min⁻¹·ml⁻¹). Slides were treated with RNase A, desalted with a final high-stringency wash (30 min) in 0.1× SSC at 60 or 75°C, dried, and apposed to Hyperfilm β-max (Amersham). Autoradiographic images were quantified using the Image-Pro Plus system. Data were manipulated using a standard curve generated from [14C] autoradiographic microscales (Amersham) and the integrated intensity of the hybridization signal was computed. For analysis of neuropeptide gene expression in subdivisions of the ARC, images were allocated at the end of the analyses of significance of different hypothalamic regions. The rostral-caudal projection of the ARC (3, 39). Data from other rodent species suggest that neuropeptide expression and were expressed throughout the ARC (Fig. 1d). Neuropeptide gene expression was also heavily concentrated in the ARC, although autoradiographic signals were also routinely detected in the LHA/perifornical region (Fig. 1e). Both MCH (Fig. 1f) and orexin mRNAs (Fig. 1g) were very strongly expressed within discrete neurons that were confined to the LHA and subthalamic areas, with similar rostral-caudal ranges. CRF gene expression was most dense in the PVH (Fig. 1h), although weaker signals were observed lateral to this nucleus in the anterior hypothalamus. There were no significant changes in MCH, orexin, or CRF gene expression with photoperiod manipulation and/or FD in experiments 1 and 2 (data not shown).

The relationship among NPY, POMC, and AGRP gene expression in ARC neurons was investigated using dual in situ hybridization. NPY and AGRP were extensively coexpressed throughout the rostral (Fig. 2a) and caudal (Fig. 2c) extents of the ARC, with silver grains (AGRP gene expression) clustered over stained neurons expressing NPY mRNA (Fig. 2e). By contrast, very few silver grains were associated with ARC neurons stained positive for POMC mRNA (Fig. 2b, d, and f), giving the appearance of a separate neuronal population.

Interaction of photoperiod and feeding state (experiment 1). Hamsters were housed in SDs for 18 wk and were either fed ad libitum throughout or were deprived of food for the final 24 h of life. Control hamsters were maintained in LDs before this treatment. Body weights fell by ~30% during 18 wk in SDs (Fig. 3) and by ~10% in both LD and SD groups when deprived of food for 24 h. The effects of photoperiod and feeding state on weights of adipose tissue and testes are shown in Table 1. Plasma insulin and cortisol concentrations are shown in Table 2. Leptin gene expression was measured by Northern blotting (Fig. 4). Overall, the effects of both SDs and FD were to reduce leptin gene expression. In RWAT (Fig. 4A), there was a significant effect of FD (P < 0.05), a trend toward an effect of photoperiod (P = 0.061), and a significant interaction effect (P < 0.05). In EWAT (Fig. 4B), there was a significant effect of photoperiod (P < 0.001) but no effect of FD. In IWK (Fig. 4C), the photoperiod effect was statistically significant (P < 0.01). In IBAT (Fig. 4D), there was an effect of photoperiod (P < 0.01), whereas FD (P = 0.08) and interaction (P = 0.063) effects approached but did not attain statistical significance.

NPY, POMC, AGRP, OB-R, and OB-Rb mRNAs were quantified by in situ hybridization in the ARC of each animal. In the relevant areas of hypothalamus, every eighth section was hybridized with each probe and quantified by image analysis. There was a significant effect of FD (P < 0.05), but not photoperiod, on OB-R gene expression (Fig. 5A). With the OB-R probe, expression of the long splice variant of the leptin receptor was shown to be regulated by both FD (P < 0.001) and photoperiod (P < 0.001; Fig. 5B); expression was reduced by maintenance in SDs and increased by FD. Neuropeptide gene expression in the ARC of the hamster was assessed both as total expres-
sion in the nucleus and as expression in the subdivisions defined earlier. There was no effect of photoperiod or FD on NPY gene expression throughout the ARC (Fig. 6A), but there was a significant increase in NPY mRNA in the caudal ARC due to FD ($P < 0.05$). POMC gene expression in the hamster ARC was significantly reduced by short photoperiod ($P < 0.05$), but there was no effect of FD (Fig. 6B). Analysis of POMC gene expression in caudal, mid-, and rostral ARC subdivisions generated the same outcomes. There were significant effects of both short photoperiod ($P < 0.05$) and FD ($P < 0.05$) on AGRP gene expression in the hamster ARC (Fig. 6C); both factors increased AGRP mRNA levels. Analysis of AGRP gene expression in caudal and rostral ARC subdivisions revealed significant increases in both caudal and rostral regions due to FD, whereas a significant effect of photoperiod was only observed in the rostral ARC.

Effect of FD in long photoperiod (experiment 2). Hamsters were divided into three groups balanced for body weight (approximately 42 g), one of which was fed ad libitum ($n = 6$). The second group was deprived of food for 24 h, giving rise to a 10% reduction in body weight ($n = 5$). The third group was deprived of food for 48 h ($n = 5$); body weight fell by 16%. Plasma insulin concentrations differed significantly between the groups ($P < 0.01$) and were reduced by FD, but there were no differences in plasma cortisol (Table 2). Group differences in leptin gene expression were observed in EWAT ($P < 0.01$; Fig. 7A) but not IWAT (Fig. 7B); FD reduced leptin mRNA levels in EWAT compared with ad libitum-fed animals.

There were no statistically significant differences between the three groups of Siberian hamsters in either OB-R or OB-Rb gene expression in the ARC (data not shown). The trend towards an increase in NPY gene expression in the ARC of food-deprived hamsters (Fig.
8A) did not reach statistical significance, an outcome that was not altered by the division of the nucleus for the purposes of analysis into caudal and rostral divisions. The opposite trend was observed for POMC gene expression in the ARC (Fig. 8B); the reduction in POMC mRNA with FD approached but did not attain statistical significance ($P = 0.11$). In the case of POMC gene expression, however, analysis of the ARC as caudal, mid-, and rostral subdivisions revealed a significant difference between groups in the caudal ARC ($P < 0.05$).

**Table 1.** Testes and adipose tissue weights in Siberian hamsters from experiment 1

<table>
<thead>
<tr>
<th>Photoperiod</th>
<th>LD Ad libitum</th>
<th>24-h FD</th>
<th>LD Ad libitum</th>
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<tr>
<td>Feeding state</td>
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<tr>
<td></td>
<td>Ad libitum</td>
<td>24-h FD</td>
<td>Ad libitum</td>
<td>24-h FD</td>
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<tr>
<td>Paired testes</td>
<td>682 ± 67†‡</td>
<td>620 ± 56†‡</td>
<td>56 ± 3</td>
<td>46 ± 3</td>
</tr>
<tr>
<td>RWAT</td>
<td>89 ± 6*</td>
<td>93 ± 7*</td>
<td>49 ± 14</td>
<td>22 ± 6</td>
</tr>
<tr>
<td>EWAT</td>
<td>903 ± 104*</td>
<td>787 ± 55*</td>
<td>347 ± 77</td>
<td>168 ± 29</td>
</tr>
<tr>
<td>IWAT</td>
<td>970 ± 120*</td>
<td>972 ± 98*</td>
<td>532 ± 123</td>
<td>339 ± 53</td>
</tr>
<tr>
<td>IBAT</td>
<td>288 ± 23*</td>
<td>215 ± 20†</td>
<td>191 ± 38†</td>
<td>98 ± 14</td>
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Values are means ± SE (in mg). LD, long-photoperiod day (16:8-h light-dark cycle); SD, short-photoperiod day (8:16-h light-dark cycle); RWAT, retroperitoneal white adipose tissue; EWAT, epididymal white adipose tissue; IWAT, inguinal white adipose tissue; IBAT, interscapular brown adipose tissue. *Different from either SD group; †different from SD food-deprived group; ‡one-way ANOVA on Ranks.
ad libitum-fed higher than 48 h FD). There were marked differences between the groups in AGRP gene expression in the ARC (P < 0.005). Gene expression was increased by both 24 and 48 h FD (Fig. 8C). Outcomes were similar when the ARC was analyzed as caudal and rostral sub-divisions. Changes in hypothalamic gene expression in experiments 1 and 2 are summarized in Table 3.

**DISCUSSION**

The distribution of leptin receptor, POMC, AGRP, MCH, and orexin mRNAs in the hamster hypothalamus (Fig. 1) was consistent with other rodent species. The strategy we adopted allowed quantification of a number of mRNA species in a nominated hypothalamic nucleus from an individual animal. This necessitated that only every eighth section was hybridized with any individual probe, and it is therefore possible that very localized changes in gene expression could be overlooked. We provide evidence of the regulation by photoperiod of POMC, AGRP, and OB-Rb gene expression in the ARC. In addition, AGRP gene expression was strongly regulated by FD, whereas more discrete effects were observed for OB-R, OB-Rb, NPY, and POMC mRNAs. It is anticipated that changes in mRNA levels will be translated into differences in peptide synthesis, as indicated in one of our earlier studies (28), and will thus be of functional significance. These changes were set against a background of changes in plasma concentrations of insulin, cortisol, and, by inference from adipose tissue mRNA, leptin.

The time points chosen for investigation in studies such as those described here are clearly important in view of the dynamics of the response to photoperiod. We chose to study the situation where body weight differences between SD and LD animals were near maximal, and it is possible that observed changes in gene expression could be specific to this particular circumstance. We studied SD hamsters that were approaching their body weight nadir but that were still on a downward trajectory (Fig. 3). It is recognized that catabolic drive in these animals may be lower than earlier in the photoperiod response. Testes weights were minimal in all SD animals and there was no evidence of spontane-

### Table 2. Plasma concentrations of insulin and cortisol in Siberian hamsters in experiments 1 and 2

<table>
<thead>
<tr>
<th></th>
<th>Insulin, µU/ml</th>
<th>Cortisol, ng/ml</th>
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<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD—ad libitum fed</td>
<td>135.78 ± 35.71</td>
<td>49.5 ± 2.1</td>
</tr>
<tr>
<td>LD—24 h food-deprived</td>
<td>19.54 ± 5.07</td>
<td>35.2 ± 5.0</td>
</tr>
<tr>
<td>LD—48 h food-deprived</td>
<td>16.35 ± 3.99</td>
<td>93.3 ± 11.3</td>
</tr>
<tr>
<td>SD—ad libitum fed</td>
<td>5.02 ± 0.57</td>
<td>85.3 ± 5.1</td>
</tr>
<tr>
<td>SD—24 h food-deprived</td>
<td>3.99 ± 0.35</td>
<td>93.3 ± 11.3</td>
</tr>
</tbody>
</table>

| **Experiment 2**     |                |                 |
| LD—ad libitum fed   | 236.4 ± 40.1  | 83.3 ± 9.3    |
| LD—24 h food deprived| 24.2 ± 2.5    | 114.1 ± 18.2 |
| LD—48 h food deprived| 19.2 ± 2.7    | 88.3 ± 9.1   |

Values are means ± SE. *Different from SD food-deprived group; †different from either SD group; ‡different from 24 h and 48 h food deprived; §one-way ANOVA on Ranks.
ous recrudescence. The gonadal regression that accompanies body weight change is also an important consideration. Further studies using castrated hamsters will be required to establish whether observed effects are directly regulated by photoperiod, or are secondary to longitudinal changes in gonadal steroid feedback. Short photoperiod also decreases POMC mRNA in the Syrian hamster (3), in which SDs induce gonadal regression but with an accompanying increase in body weight (1).

It will also be necessary to establish whether photoperiod-induced changes in specific regulatory systems are relevant to energy balance.

The role of the leptin system in SD-induced body weight reduction was assessed through study of leptin mRNA in adipose tissue and leptin receptor mRNA in the ARC. Eighteen weeks in SDs depressed leptin mRNA in adipose tissues (Fig. 4), corroborating data recorded elsewhere (18). Thus leptin gene expression was positively related to adipose tissue weight in ad libitum-fed LD and SD hamsters, and it is likely that plasma leptin levels fall as a consequence of weight loss and adipose tissue mobilization. There was no evidence that seasonal weight loss was the direct result of an increase in leptin levels, as might be anticipated if leptin was a critical hormone inhibiting food intake and decreasing body weight in proportion to its circulating concentration. Data from other rodent models indicate that acute energetic manipulations such as FD and cold exposure reduce leptin gene expression and plasma leptin in the absence of substantial changes in adipose tissue weight. These changes, in turn, upregulate receptor expression (2, 21, 30). The data from the food-deprived hamster support this contention. Increases in leptin receptor mRNA on FD were more pronounced in SDs than LDs, although there was no evidence that the already low level of leptin mRNA in adipose tissue of SD hamsters could be further downregulated by FD (Fig. 4). Leptin gene expression in the SD hamster may be at a “basal” level. Although, by inference, SDs reduced plasma leptin; OB-Rb gene expression in the ARC was also decreased in these animals (Fig. 5). Expression of the OB-Rb mRNA appeared to be more sensitive to photoperiodic regulation than OB-R (Fig. 5), supporting the hypothesis that OB-Rb is the predominant regulated splice variant (2, 30).

The foregoing discussion argues that leptin regulates the transcription of its own receptor and, furthermore,
suggests that leptin receptor gene expression is differentially regulated by acute and chronic changes in ligand availability. This may have bearing on the paradoxical nature of the leptin signal in animals expressing seasonal cycles of body weight and adiposity (23). How does leptin contribute to the regulation of body weight in the seasonal animal while apparently reflecting the overall level of adiposity without acting to reverse the changes programmed by photoperiod? Elevated leptin receptor gene expression may contribute to an increase in sensitivity to leptin (2). Thus reduced OB-Rb expression in SDs implies that increased sensitivity to leptin is also unlikely to have a causative role in SD weight loss. Furthermore, reduced receptor expression could alter the downstream consequences of declining leptin feedback in SDs and represent a mechanism for disengaging this signal. It remains to be determined how chronic incremental changes in plasma leptin are integrated into hypothalamic regulatory systems.

Expression levels of POMC and AGRP mRNA in the ARC of the Siberian hamster were down- and upregulated, respectively, after 18 wk in SDs (Fig. 6, B and C). These changes were not consistent with a critical role for these peptides in photoperiod-induced body weight loss based on our knowledge of the function of these peptides in other rodent models. The reduction in POMC gene expression in SDs presumably leads to a lower rate of synthesis of the POMC polypeptide, a precursor that undergoes posttranslational processing to generate a number of products, including the MCs. MCs, such as α-melanocyte-stimulating hormone (α-MSH), are potent anorexics on activation of MC4 receptor, and targeted deletion of the latter causes severe obesity (16). The activity of the POMC/MC receptor system is thus of interest in the context of the body weight cycles expressed by the Siberian hamster, in which the majority of weight change is due to variation in body adiposity (43). Agouti protein is an antagonist of MC receptors and causes obesity when ectopically expressed in the hypothalamus (11). AGRP, a homologue of agouti protein, acts as an endogenous antagonist of the MC4 and MC3 receptors, thereby exerting an anabolic effect (33). Accordingly, overexpression of AGRP in transgenic mice produces an obese phenotype very similar to the MC4-receptor knockout animal (33). AGRP gene expression was elevated in SD hamsters (Fig. 6C). Increased production of this MC4-receptor antagonist would accentuate the effect of reduced MC (POMC) synthesis. The summated effect of these changes would therefore be to reduce the negative drive on energy balance through the MC4 receptor and presumably oppose the programmed catabolic state that exists in the SD hamster.

AGRP mRNA in the hamster ARC was upregulated by FD as well as by short photoperiod. These effects in the hamster were consistent with mouse studies in which fasting increased AGRP mRNA (31). In the mouse, AGRP and NPY respond similarly to fasting. It is noteworthy that in adjacent sections of hamster...
MCH mRNA was upregulated by fasting in mice (34), on intracerebroventricular administration (34, 36). Hamster. Orexin and MCH stimulate food intake in rats regulated by either photoperiod or FD in the Siberian that expression of CRF, MCH, or orexin mRNA was restricted to the caudal portion of the nucleus. Interestingly, the effect of photoperiod on AGRP and other neuronal phenotypes in the ARC is an important objective. Our coexpression studies (Fig. 2) indicated that a population of ARC neurons is able to synthesize both an agonist for an anabolic neuroendocrine system (NPY) and an antagonist for a catabolic system (AGRP). By contrast, POMC-expressing neurons appeared to form a separate population. Indeed, recent evidence suggests that POMC mRNA is coexpressed in arcuate neurons with mRNA for another anorectic peptide, cocaine- and amphetamine-regulated transcript (9, 19). In light of our coexpression evidence, the differential regulation of NPY and AGRP mRNAs by photoperiod and FD is strongly suggestive of transcript-specific regulation, possibly by leptin, at the level of individual neurons.

There is growing evidence from studies of other rodent species that arcuate neurons expressing a particular neuropeptide mRNA may exist as different functional populations along the rostral-caudal extent of the nucleus (3, 39, and references therein). In the Syrian hamster (3), the rostral ARC appeared to be the most sensitive to photoperiod when POMC gene expression was assessed, although similar effects were observed throughout the nucleus. In the Siberian hamster, there were significant effects of photoperiod on POMC mRNA in all three defined subdivisions of the ARC. Interestingly, the effect of photoperiod on AGRP gene expression was confined to the rostral ARC. When the effects of FD were examined, there was no apparent regional difference in response of AGRP gene expression, whereas changes in POMC and NPY gene expression were restricted to the caudal portion of the nucleus. This finding contrasts with data for POMC and NPY gene expression in the laboratory rat, suggesting that the rostral ARC exhibits regional sensitivity to FD.

There was no evidence from the current experiments that expression of CRF, MCH, or orexin mRNA was regulated by either photoperiod or FD in the Siberian hamster. Orexin and MCH stimulate food intake in rats on intracerebroventricular administration (34, 36). MCH mRNA was upregulated by fasting in mice (34), but it has been suggested that this peptide might not be involved in the regulation of body weight, because repeated intracerebroventricular administration did not lead to weight gain (35). Targeted deletion of the MCH gene, however, gave rise to mice with a hypophagic and lean phenotype (40), suggestive of a more critical regulatory role for the peptide. Orexin (also known as hypocretin) (8) gene expression was increased by FD in rats (36). The MCH and orexin gene expression data from the food-deprived Siberian hamster and suggestions that orexin peptides may have a limited effect on food intake in the mouse (22) lend further support to the idea of species specificity in networks regulating energy homeostasis. Further interpretation of neuroendocrine responses to photoperiod and FD in the Siberian hamster must await in vivo investigations, because, with the exception of NPY (4), none of the putative mediators of energy balance, including leptin, have been tested by intracerebroventricular injection in this species.

Recent progress has identified a number of new neurochemicals that are involved in energy homeostasis. Rodent models of long-term regulated change in body weight and adiposity, such as the Siberian hamster, provide an appropriate background against which to assess the relative contributions of different signaling systems within a physiological context. One of the major challenges presented by these models is to distinguish neuroendocrine changes that drive the dynamic weight change from those that are secondary to other changes or to other coincident physiological changes. It should be noted that neuroendocrine systems that are involved in short-term regulation, and that might thus be perturbed by imposed energetic manipulations, need not necessarily show changes in activity in response to seasonally appropriate body weight change (23). The changes that we detected in POMC and AGRP gene expression were counterintuitive with respect to the proposed involvement of these peptides in the regulation of food intake and body weight. The expression of these hypothalamic mRNAs and that of the leptin and MC receptors needs to be examined at different stages of the weight change dynamic and in the absence of accompanying changes in gonadal steroid. This will establish whether changes are incremental with duration of exposure to SDs, and thus with body weight change, or whether there is a more abrupt switch in gene expression. The latter circumstance might highlight systems that are involved in programming body weight change. The function of leptin in seasonal body weight regulation is unknown, and it seems unlikely that leptin is directly responsible for weight loss in short photoperiod. Rather, changes in leptin gene expression and plasma leptin are probably a consequence of this weight loss. The reduction in leptin receptor gene expression coincident with low body weight and reduced leptin synthesis may enable programmed weight change to be effected in the presence of a potentially counteracting low leptin signal.
Perspectives

Our knowledge of the different signaling pathways that are involved in the control of food intake or that are activated by energy imbalance has expanded considerably in the last few years. Although we know some details of the pathways that are activated to defend body weight against imposed negative energy balance, or that are perturbed in obesity, the detailed mechanistic underpinnings of physiological body weight regulation are poorly understood. Siberian hamsters are able to encode and defend a shifting seasonally appropriate body weight from further imposed change (41). Regulated dynamic body weight change and changes in adiposity must require some reprogramming of the hypothalamus both in terms of the way inputs such as leptin are integrated and in terms of output to other central nervous system centers. The mechanisms controlling these processes are likely to be applicable in most mammalian species, including humans. For example, the means by which chronic incremental changes in leptin signaling are integrated into hypothalamic regulatory systems may provide insight into the development of human obesity. Similarly, the gradual resetting in an upward direction of the body weight that will be defended may be at the root of the difficulty experienced by many individuals in sustaining weight loss achieved by dieting.

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Address for reprint requests and other correspondence: J. Mercer, Molecular Neuroendocrinology Unit, Rowett Research Institute, Aberdeen AB21 9SB, UK (E-mail: jgm@ri.sari.ac.uk).

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