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Neuropeptides and anticipatory changes in behaviour and physiology: seasonal body weight regulation in the Siberian hamster

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Abstract

The Siberian hamster, *Phodopus sungorus*, is a powerful model of physiological body weight regulation. This seasonal model offers the potential to distinguish between the compensatory neuroendocrine systems that defend body weight against imposed negative energy balance, and those that are involved in the programming of the level of body weight that will be defended—a seasonally appropriate body weight. Of the known, studied, components of the hypothalamic energy balance system, the anorexogenic peptide, cocaine- and amphetamine-regulated transcript (CART), is the only candidate where gene expression changes in a manner consistent with a role in initiating or sustaining photoperiod-induced differences in body weight trajectory. Siberian hamsters effect a reversible biannual switch in leptin sensitivity in which only short day (SD)-acclimated hamsters that have undergone a reduction in body weight, adiposity and plasma leptin are sensitive to peripheral exogenous leptin. The suppressor of cytokine signalling protein, SOCS3, appears to be the molecular correlate of this seasonal sensitivity.

Keywords: Melatonin; Photoperiod; *Phodopus*; Leptin; SOCS3; CART

1. Anticipating a predictably hostile environment

A significant proportion of mammalian species lives out their lives in environments that not only vary from day-to-day and within each 24-h period, but which also have a pronounced seasonal cycle. Among the primary environmental variables to which animals have to adapt are day length, temperature, and climatic conditions such as rainfall and windspeed. These primary variables in turn have a major influence on food supply, energy expenditure and thus on the probability of successful breeding outcome. Consequently, animals that live in temperate latitudes are profoundly seasonal, an attribute perhaps best exemplified by the compression of reproductive and breeding behaviour and physiology into particular parts of the year. Since, in temperate latitudes, the seasons are predictable in occurrence, if not entirely in severity or duration, the possibility is opened up for animals to prepare their physiological processes for the coming challenges. By undertaking anticipatory changes in behaviour and physiology, animals are less likely to be caught out by seasonal changes in climate that might affect their own survival, and are less likely to invest costly effort in gestating and rearing young whose own survival prospects are limited by the environment into which they are born.

In order to be able to alter behaviour and physiology in anticipation of seasonal change, suitable environmental cues must be perceived and correctly interpreted before the animal commits itself to potentially costly adaptations. The environmental cue that is most predictable, and least likely to be subject to short-term or year-on-year variation, is day length or photoperiod, whereas another environmental cue, ambient temperature, is relatively undamped in terms of its day-to-day variability. Using the photoperiod signal, seasonal mammals synchronise a number of different key physiologies to the seasonal cycle in climate and thus in availability of nutrients. Characteristically, mating is timed in coordination with the gestation period of the species in question in order that offspring are born into a favourable

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environment where food is plentiful. However, in addition to seasonal reproductive cycles, such species frequently display additional physiological adaptations to the environment. Of particular interest in the context of this review are the annual cycles of food intake and body weight (Mercer, 1998). All animals consume food to satisfy their requirement for energy and nutrients. These requirements are determined by the physiological state of the animal at any given time, but, by the same token, the availability of food is a major determinant of the physiological state that the animal can afford to maintain. Mammals that live in temperate latitudes are variously confronted by a shortage or complete absence of food during the winter months, and have adapted their physiology and behaviour accordingly. These animals clearly *anticipate* the change in food availability and adopt strategies to cope with and survive this challenge. A familiar example of this is hibernation, wherein fat stores are laid down during times of plenty, and the animal lives off this stored energy during the winter months, when food is essentially unavailable, at least in the environment outside the overwinter retreat. Less familiar, but nevertheless with proven evolutionary value, is the strategy of voluntarily reducing food intake and body weight during the transition between summer and winter photoperiods. The strength of this drive to adhere to a seasonally appropriate body weight trajectory is illustrated by two rather different mammalian species, the Siberian hamster, *Phodopus sungorus*, also known as the Djungarian hamster, and the sheep, both of which, despite the provision of food in excess in the laboratory throughout the year, increase food intake and body weight in long photoperiods, and decrease food intake and body weight in short photoperiods.

2. Seasonal body weight regulation in the Siberian hamster

The changes induced by natural, and gradually changing photoperiod cues can be replicated in the laboratory by a simple square-wave switch in photoperiod. Many small seasonal mammals such as the Siberian hamster exhibit profound anticipatory changes in food intake, body weight and adiposity in response such simple changes in photoperiod (Wade and Bartness, 1984; Morgan and Mercer, 2001). Thus, transfer of laboratory-reared adult male hamsters from long day (LD) photoperiod (16 h light/8 h dark) to short day (SD) photoperiod (8 h light/16 h dark) can induce weight loss that may average 30–40% over a 12–18-week period (Fig. 1; Mercer et al., 2000, 2001). These large amplitude changes in body weight are reversible, either spontaneously following the development of a refractory state in hamsters held in SDs for prolonged periods, or following transfer back to LDs. Similarly, juvenile hamsters are also sensitive to photoperiod; transfer to SDs at weaning giving rise to restricted growth, low body fat and delayed pubertal development (Adam et al., 2000). Over the last two decades, and particularly since the landmark cloning of the leptin gene, rapid progress has been made in identifying components of the hypothalamic energy balance circuitry and peripheral feedback to these circuits (Schwartz et al., 2000; Woods et al., 1998) in defining the involvement of molecular components in the compensatory response of rodents to imposed challenges such as negative energy balance. The hamster model serves to emphasise the difference between compensatory and programmed body weight regulation, the former being essentially a defence mechanism, whereas the latter provides a means of effecting advantageous long-term changes in body weight, and moreover in the level of body weight that will be defended (Morgan and Mercer, 2001; Mercer and Speakman, 2001). By contrast to the compensatory systems, the regulation of programmed or anticipatory body weight change, such as that exhibited by seasonal mammals, remains largely unresolved, and elucidation of the mechanisms by which ‘defended body weight’ is adjusted is a research objective of considerable significance.

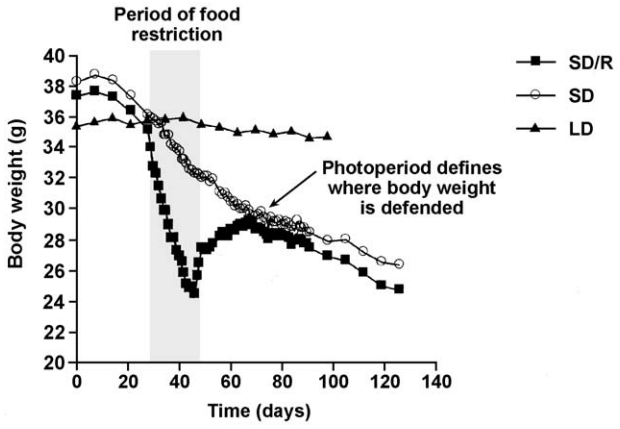

Fig. 1. Body weight of male Siberian hamsters fed ad libitum in short day length (SD) for 126 days, or held in short day length with restricted food (60% of ad libitum intake) between days 28 and 46 (SD/R). Shaded area represents food restriction period. For comparison, a typical body weight trajectory of hamsters fed ad libitum in long day length is shown (LD).

The power of the Siberian hamster as a model in which to address the issues outlined above is exemplified by a comparatively simple experiment that provides some of the best evidence that mammals directly regulate their body weight, and also provides some insight into how different levels of body weight regulation might function. The characteristics of body weight regulation in this species suggest the existence of a comparator system whereby actual body composition is assessed against encoded seasonally appropriate ‘target’ parameters. The behaviour of this system is defined in experiments first described over 20 years ago, where food restriction was superimposed on weight loss induced by a natural shortening photoperiod (Steinlechner et al., 1983), causing body weight to fall below a seasonally appropriate level. We

have recently replicated this experiment in the laboratory with a square-wave photoperiod transformation, with essentially the same outcome (Mercer et al., 2001). When restriction is lifted, body weight increases but only to the point where it approximates to the declining weight of control animals fed ad libitum in SDs throughout. The previously restricted animals then adopt a weight trajectory that parallels that of the control group (Fig. 1). Thus, the system behaves in a manner consistent with the seasonal timekeeping mechanism continuing to operate, and to adjust the encoded appropriate body weight, even when animals are prevented from maintaining their desired body weight (Bartness et al., 1989). There are several lines of evidence to suggest that melatonin signal 'accumulates', presumably at a brain site; the entry of animals into a refractory state appears to be determined by the number of days during which the nocturnal melatonin secretion profile exceeds a certain threshold, while the restriction experiment depicted in Fig. 1 is suggestive of incremental changes in appropriate body weight according to the accumulating photoperiodic history of the animal.

3. Central and peripheral energy balance systems

The maintenance of an appropriate body weight involves interactions between a network of central and peripheral signalling systems focussed on critical integratory centres in the hypothalamus (Kalra et al., 1999). The cloning of the leptin gene in 1994 (Zhang et al., 1994) has been the catalyst for increased activity in the field of energy balance, and several new candidate hypothalamic neuropeptide and receptor systems have been implicated in the regulation of food intake and body weight. A primary role for leptin is in the communication of information about adipose tissue energy stores and energy flux, providing prompt feedback to brain centres involved in the regulation of energy balance (Ahima and Flier, 2000). Leptin is generally present in the circulation in proportion to body adiposity, and exogenously administered leptin reduces food intake and body weight. The primary brain target of the leptin signal appears to be the hypothalamic arcuate nucleus, although other hypothalamic structures such as the dorsomedial nucleus, the lateral hypothalamus and the paraventricular hypothalamic nucleus also express the leptin receptor (Mercer et al., 1996). It is now recognised that the arcuate nucleus contains complementary orexigenic (e.g. neuropeptide Y and agouti-related protein [AGRP]; Ollmann et al., 1997) and anorexigenic (e.g. proopiomelanocortin [POMC] and cocaine- and amphetamine-regulated transcript [CART]) neurones that target the paraventricular hypothalamic nucleus. The lateral hypothalamus, which contains the cell bodies of the melanin concentrating hormone (MCH; Qu et al., 1996) and orexin (de Lecca et al., 1998; Sakurai et al., 1998) orexigenic systems, has connections to and from the arcuate, and has long been considered an important site in energy balance. Elucidation of the interactions of different components of the signalling array in the context of physiological body weight regulation has not been well studied, and may provide insight into the longer-term regulation of body weight in the normal animal. The majority of the information that we possess about the above systems relates to their involvement in the 'defence' of body weight against energy deficit. By contrast, little is known of the signalling framework underlying the encoding of an 'appropriate' body weight, i.e. the determination of the level at which body weight will be defended. Experimental evidence and mammalian life histories indicate that body weight regulation does indeed function at different levels (Morgan and Mercer, 2001; Mercer and Speakman, 2001). These can be broadly categorised as 'compensatory' weight change (i.e. acting to reverse an imposed perturbation) and 'programmed' long-term weight control, including anticipatory weight change.

Seasonal body weight trajectories have the appearance of being tightly controlled. There are a number of plausible routes through which photoperiod and the pineal hormone, melatonin, could effect this regulation (Fig. 2). Photoperiod could alter the tone of orexigenic and/or anorexigenic drive within the hypothalamus, modulate sensitivity to peripheral hormonal inputs, and in particular sensitivity to leptin, or it could impact upon as yet unknown regulatory systems that are relatively elevated in the hierarchy of energy balance signalling, i.e. that bridge the gap in our knowledge between the durational melatonin signal and the compensatory hypothalamic systems. In surveying known pathways for evidence of involvement in seasonal body weight trajectories or in attempts to define novel components of the regulatory system, we could be looking for either gradual, incremental changes in the activity of a signalling system that leads body weight along an appropriate course, or for a more abrupt switch in activity that effectively pushes body weight along. In examining neuroendocrine systems that are involved in short-term 'compensatory' regulation, and that are perturbed by imposed energetic manipulations, we should anticipate that many of these systems will not change activity in response to seasonally appropriate body weight change, and it will be important to distinguish neuroendo-

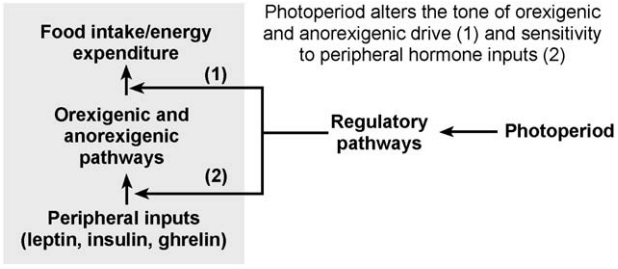

Fig. 2. Schematic showing possible mechanisms of seasonal body weight regulation in the Siberian hamster.

crine changes that drive the dynamic weight change from those that are secondary either to this change or to other co-incident physiological changes. In studying the molecular mechanisms underlying seasonal weight change, the majority of studies have understandably concentrated on animals with established body weight differentials. Latterly, however, with the identification of some potential mediators of seasonal weight change, attention has turned to the more dynamic phase in the seasonal cycle as body weight trajectories begin to diverge.

4. Hypothalamic neuropeptides in the Siberian hamster

Studies in a number of laboratories carrying out research with the Siberian hamster have identified several neuropeptide and receptor genes where levels of expression change following photoperiod manipulation. Significantly, compared with their respective controls, profiles of hypothalamic gene expression in adult male hamsters exhibiting sustained SD weight loss were very similar to those of SD juvenile females with growth restriction (Mercer et al., 2000, 2001; Adam et al., 2000). Changes are centred on the arcuate nucleus. The common denominator in these studies is POMC, the mRNA of which is consistently down-regulated after exposure to SDs of a duration sufficient to induce significant weight differential (Reddy et al., 1999; Mercer et al., 2000, 2001; Adam et al., 2000; Rousseau et al., 2002). This down-regulation is observed in both adult and juvenile animals after prolonged SD exposure but not after exposures of 3 weeks or less (Mercer et al., 2003). The counter-intuitive down-regulation of POMC gene expression, which would presumably result in reduced catabolic drive through the melanocortin MC_4 receptor in the paraventricular hypothalamic nucleus, has been shown to be photoperiodically driven and not to be influenced by declining gonadal steroid in elegant steroid clamped castrate experiments carried out by Rousseau et al. (2002). This is important since one component of the physiological response of the Siberian hamster to short photoperiod is the regression of the reproductive system and an accompanying fall in gonadal steroid synthesis. The reduction in gonadal steroid feedback will be responsible for part of the weight loss observed on transfer to SDs since surgical gonadectomy reduces body weight in LD hamsters independent of any photoperiod change (Wade and Bartness, 1984; Mercer et al., 1997). Other neuropeptide genes, which have shown altered expression following photoperiod manipulation, include AGRP and CART. Significantly, neuropeptide Y gene expression has been consistently demonstrated not to be affected by the prevailing photoperiod even when hamsters at their SD body weight nadir are compared to maximum weight LD animals (Reddy et al., 1999; Mercer et al., 2000, 2001; Adam et al., 2000). The neuropeptide Y gene has been well characterised as one of the orexigenic systems that are up-regulated in negative energy balance states such as food deprivation and its stability throughout photoperiod-induced weight change is interpreted as recognition of an appropriate state of energy balance despite a net catabolic state, i.e. seasonally appropriate weight change. The changes observed in AGRP gene expression in adult males (Mercer et al., 2000) were again counter-intuitive in the context of weight loss - elevated expression being likely, if translated into peptide synthesis, to increase antagonist availability at the melanocortin MC_4 receptor and thus further reduce catabolic drive. However, trends in the AGRP data from juvenile female hamsters were in the opposite direction (Adam et al., 2000), so the significance of these findings is unclear. Nevertheless, overall the changes in POMC and AGRP gene expression were not consistent with a critical role for signalling through the melanocortin MC_4 receptor in photoperiod-induced body weight trajectories, since their summated effect would be to reduce the negative drive on energy balance and presumably oppose the programmed catabolic state that exists in the SD hamster. Of relevance, although a melanocortin MC_4 receptor agonist administered into the cerebral ventricle of the brain inhibits food intake in Siberian hamsters, there was no effect of photoperiod on sensitivity to this compound (Schuhler et al., 2003). Neuropeptide Y is similarly equipotent in LD and SD hamsters (Boss-Williams and Bartness, 1996).

In the case of CART, observed changes in SDs were in the direction that would be anticipated for a catabolic peptide (Kristensen et al., 1998) that was involved in the establishment of a body weight differential. In our laboratory, and in animals drawn from the Rowett breeding colony, the up-regulation of CART gene expression in the arcuate nucleus is very consistent, being observed following a number of different manipulations of adult and juvenile hamsters of either sex, while, most significantly, these changes were observed as early as 2 weeks into SD exposure and prior to the development of a significant LD–SD weight differential (Adam et al., 2000; Mercer et al., 2003). This suggests not only a role for this catabolic/anorexigenic peptide in SD weight loss or growth restriction, but also that a change in activity of the CART system could be involved in driving seasonal body weight regulation, as opposed to being a secondary response to that regulation. However, other laboratories have been unable to substantiate these findings. Possible explanations for this situation have been discussed in a number of recent publications (Rousseau et al., 2002; Robson et al., 2002; Mercer et al., 2003), without resolution, although from comparison of the studies of Rousseau et al. (2002) and Robson et al. (2002), gonadal steroid status appears unlikely to provide an explanation. Technical differences between the studies could be important, but these could be difficult to unravel.

In order to distinguish the effect of photoperiod on hypothalamic gene expression from the effect of the weight loss that accompanies prolonged SD exposure, i.e. to differentiate between hypothalamic changes that

induce weight change and those that are secondary, consequential, events, hamsters held in LDs were food restricted to mimic the body weight trajectory of SD hamsters (Mercer et al., 2001). Although an equivalent degree of weight loss could be achieved through complete food deprivation, this is an inappropriate manipulation for comparison with short photoperiod-induced weight reduction. The more subtle manipulation of food intake through long-term restriction is likely to be more informative. The response of adult male hamsters to this imposed negative energy balance was largely as anticipated from studies of other rodents, with orexigenic systems and anorexigenic systems being, respectively, up- and down-regulated, consistent with their involvement in body weight defence or compensatory processes. Two of the genes examined were regulated in opposite directions by a similar degree of weight loss achieved through either imposed or programmed weight change achieved through energy restriction or photoperiod (Fig. 3). These genes were CART and the leptin receptor long form. CART gene expression, predictably, was down-regulated by imposed negative energy balance, but as discussed above was up-regulated in SDs. The opposite effects were observed for leptin receptor long form; low leptin levels induced by food restriction in LDs resulted in an up-regulation of receptor expression, in line with predictions from non-seasonal rodents (Baskin et al., 1998), whereas in SDs, leptin receptor long form gene expression was reduced compared to LD controls, as reported previously (Mercer et al., 2000). These findings clearly suggest some modulation of the way the leptin signal is read and integrated into the hypothalamus, according to the photoperiodic history of the animal, facilitating the recognition of the difference between weight change brought about by imposed negative energy balance and seasonal, and thus by definition appropriate, body weight change.

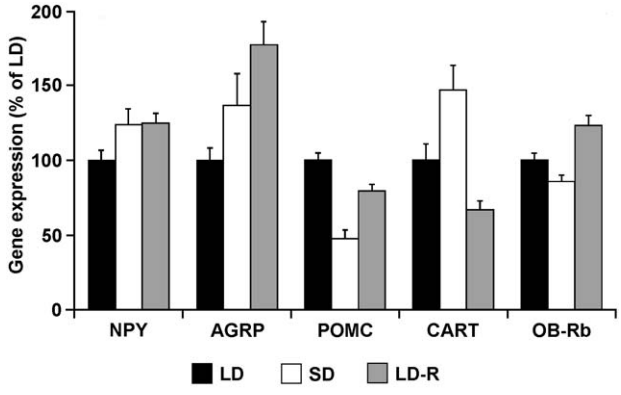

Fig. 3. Neuropeptide and leptin receptor gene expression in the hypothalamic arcuate nucleus of adult male Siberian hamsters ($n=9$ or 10) fed ad libitum in long (LD) or short day length (SD) for 84 days, or held in long day length with restricted food from day 14 onwards to mimic short day length body weight trajectory (LD-R). Values are expressed as percentages of values in LD-ADLIB hamsters. Means ± S.E.M. Abbreviations: neuropeptide Y, NPY; agouti-related protein, AGRP; proopiomelanocortin, POMC; cocaine and amphetamine-regulated transcript, CART; leptin receptor long form, OB-Rb.

5. Leptin signalling in the Siberian hamster

The concept of differential integration of the leptin signal into the hypothalamic circuitry was identified earlier as one of the routes through which mammals might effect changes in behaviour and physiology in response to a changing environment (Fig. 2), i.e. through adjustments in sensitivity to peripheral hormonal feedback. In the adult male Siberian hamster, a significant proportion of the body weight lost during the transition from an obese, LD phenotype to a lean, SD phenotype is adipose tissue. It is now well established that mobilisation of adipose tissue in SD hamsters reduces leptin gene expression and blood leptin concentrations (Klingenspor et al., 1996, 2000). The paradox in this feedback, then, is why, given the known effects of leptin on energy balance, the declining leptin signal during this transition does not act to reverse the changes programmed by photoperiod. The explanation of this paradox is now emerging from the data generated in a number of functional and molecular studies, although precisely what information is communicated in the leptin signal in seasonal mammals is still a matter for debate. For example, feedback on the levels of body fat storage is likely to be read quite differently to acute changes that result from energetic challenges, to which the lean SD hamster is likely to be more alert.

A number of studies of exogenous leptin administration to Siberian hamsters either by injection or by infusion with osmotic minipumps provide support for seasonal changes in sensitivity to leptin. Thus, LD and SD male hamsters had reduced food intake and body weight in response to peripheral injections of leptin over a 10-day period, but the effect on body weight was more substantial in SDs (Klingenspor et al., 2000). Using 14-day continuous infusions of recombinant leptin into male and ovariectomised oestradiol-replaced female hamsters, Atcha et al. (2000) observed reductions in body weight and adiposity in SD hamsters, but not in LD controls. There was no effect of leptin on food intake in either photoperiod. These seasonal changes in sensitivity to leptin, comparing relatively obese, LD hamsters with lean, SD hamsters are reminiscent of leptin resistance in age-related and diet-induced obesity (Scarpace et al., 2002), where experimentally reversing the trend towards increasing adiposity and hyperleptinaemia overcomes the insensitive state. However, experimental evidence suggests that leptin insensitivity in the LD hamster is not driven directly through body fat and circulating leptin, since LD hamsters that were food restricted prior to leptin infusion and thus had low plasma leptin and depleted fat depots remained insensitive, whereas SD animals with similar body composition and circu-

lating leptin lost further body weight and fat (Rousseau et al., 2002). These data clearly imply that it is photoperiod rather than nutritional or leptin status that is the key regulator of leptin sensitivity in the seasonal model. Regulated sensitivity to leptin feedback may be critical for the maintenance of seasonal bodyweight.

Insensitivity to leptin in LD Siberian hamsters could be the result of changes at a number of levels in the signalling pathway from molecule to post-receptor signal transduction. The rate of entry of leptin into the brain across the blood–brain barrier could be reduced. Alternatively, there could be a reduction in the availability of leptin receptors on the cell surface, although the elevated mRNA levels in LD hamsters compared to SD hamsters makes this appear unlikely (Fig. 3). More plausibly, there may be down-regulation of intracellular signal transduction distal to the leptin receptor. The JAK/STAT signalling pathway plays a critical role in mediating the effects of leptin on intracellular signalling (Sweeney, 2002). The Janus-family tyrosine kinases (JAKs) phosphorylate STAT proteins (signal transducers and activators of transcription), specifically STAT3, which then facilitates transcription of target genes. One class of target genes is the suppressors of cytokine signalling (SOCS), including SOCS3, which inhibits JAK/STAT activity and subsequent signal transduction. Thus, leptin activates SOCS3, which then reduces intracellular signalling by inhibiting the JAK/STAT pathway. This signalling system thus possesses powerful feedback loops, changes in the activity of which could well underlie observed changes in leptin responsiveness in the seasonal hamster. Such regulation may underlie leptin resistance in ageing-related obesity (Scarpace and Turner, 2001).

Our preliminary data from analysis of SOCS3 gene expression in the Siberian hamster do indeed suggest a role for this peptide in the reversible changes in sensitivity to leptin. SOCS3 in the arcuate nucleus is up-regulated in LDs compared to SDs (Tups, A., Ellis, C., Mercer, J.G., unpublished results). In juvenile female hamsters, this gene expression differential is apparent quite rapidly after photoperiod manipulation, and this appears to be a photoperiod effect rather than one that is dependent upon changes in body adiposity or leptin levels, thereby providing clear parallels with the in vivo functional data described above. Furthermore, SOCS3 mRNA in the arcuate nucleus was acutely stimulated by peripheral leptin injection only in SDs and not in LDs. Thus SOCS3 constitutes a molecular correlate of whole animal leptin sensitivity. These findings support the notion that reduced SOCS3 activity contributes to the increased sensitivity to leptin in SDs and, conversely, that increased SOCS3 activity results in the relative leptin insensitivity seen in LD.

One interpretation of these data is that the changing photoperiod primes arcuate nucleus sensitivity to circulating leptin, again suggestive of a direct interaction between the leptin and melatonin systems. This provides an explanation for how broadly equivalent long-term changes in the circulating leptin signal may be read differently according to the manipulation employed in their generation. The apparent early change in sensitivity at least at a molecular level could be interpreted as the leptin signal contributing to the maintenance of an appropriate body weight trajectory in SDs.

6. The role of the hypothalamic arcuate nucleus

The arcuate nucleus is a common denominator for the two genes, CART and SOCS3, that exhibit changes in gene expression prior to or simultaneous with the divergence of body weight trajectories in LDs and SDs. However, doubts exist surrounding the likely role of the arcuate nucleus in seasonal body weight cycles in the light of recent studies of hamsters bearing monosodium glutamate lesions (Ebling et al., 1998). Monosodium glutamate-lesioned Siberian hamsters, although growing more slowly than controls, retained their ability to regulate body weight to a seasonally appropriate level, despite the loss of 74% of neuropeptide Y gene expression in the arcuate nucleus in adult life, as a consequence of neonatal monosodium glutamate treatment. Although this is a major lesion, and one that also affects other brain regions that are considered to be outside the blood–brain barrier, it is possible that surviving neuropeptide Y-ergic cells still possess regulatory potential. The effect of monosodium glutamate treatment on other neuronal phenotypes in these animals is unknown. However, caution should be exercised before the potential role of a hypothalamic structure with such a pivotal function in peripheral-brain communication is discounted. Changes in the arcuate nucleus could be involved in effecting the adjustments in body composition that are required for the maintenance of an appropriate body weight, the level of which is likely to be encoded in a brain centre that is either directly or indirectly sensitive to seasonal inputs, photoperiod and the melatonin signal.

7. Resetting a seasonally appropriate body weight

The behaviour of the seasonal body weight regulatory process in Siberian hamsters is indicative of a brain system that somehow encodes a target body weight or body composition, and a comparator that allows actual body weight to be compared to this target. The accumulating melatonin signal then effects adjustments in this target that are appropriate to the perceived season. The known regulators of energy balance may be involved in executing adjustments in actual body weight but seem unlikely to be directly involved in the progressive resetting of the target or defended body weight. It is likely that components of the regulatory system are still to be discovered, particularly those that are relatively elevated in the hierarchy of signalling and which may be close to the integration of

the melatonin signal into the regulatory process. It is reasonable to assume that genes that are involved in determining the seasonal target body weight will exhibit maximal differences in expression at the extremes of body weight in LDs and SDs. Using this rationale, we have employed subtractive hybridisation techniques and gene arrays to identify genes that change in expression in response to photoperiod manipulation. The task for the future is to demonstrate that leads so generated are causative of weight change rather than secondary to that change. Identification of key components of a system that determines the level of body weight that will be defended, as opposed to the system that is involved in that defence, would open up a new class of targets for pharmacological manipulation. There is clear potential for such genes as drug targets in human obesity, where elevated body weight is strongly defended against conventional attempts at weight loss.

8. Summary

Although we understand some of the details of the pathways that are activated to defend body weight against imposed negative energy balance, or which are perturbed in obesity, the detailed mechanistic underpinnings of physiological body weight regulation are poorly understood. The accumulating data suggest that changes in signal transduction through the leptin receptor, mediated by SOCS3, and in the activity of the anorexigenic hypothalamic peptide, CART may be involved in the chain of events that leads to physiological changes in body weight in the Siberian hamster induced by photoperiod manipulation. Indeed CART is the only candidate mediator of SD weight loss whose activity changed in a manner consistent with the observed catabolic weight loss or growth restriction state, and which exhibited changes at or prior to the divergence of body weight trajectories in different photoperiods. Even so, changes in CART gene expression could clearly be responsive rather than inductive. Establishing a direct causative role in seasonal body weight regulation for any putative component of the signalling system represents a substantial challenge. Siberian hamsters are able to encode and defend a shifting seasonally appropriate body weight from further imposed change. The mechanisms controlling these processes are likely to be applicable in most mammalian species, including man, although not in a seasonal context. For example, the means by which chronic incremental changes in leptin signalling are integrated into hypothalamic regulatory systems may provide insight into the development of leptin resistance in human obesity. Similarly, the gradual resetting in an upward direction of the body weight that will be defended may underlie the difficulty experienced by many individuals in sustaining weight loss achieved by dieting. Seasonal animal models such as the Siberian hamster, where defended body weight is readily manipulated by photoperiod, provide a route through to these mechanisms.

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