Photoperiodic regulation of insulin receptor mRNA and intracellular insulin signaling in the arcuate nucleus of the Siberian hamster, *Phodopus sungorus*

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Adiposity signals (30). The seasonal cycle in body weight is associated with a correlation of these hormones in proportion to body fat mass. Leptin and insulin are transported into the brain from the bloodstream where they bind to their receptors and, in particular, those in the arcuate nucleus, a key neuronal center for control of energy homeostasis. Activation of signal transduction pathways distal to their receptors integrates these peripheral signals into a neuronal response. Leptin and insulin exhibit similarities in their central effects (both display catabolic actions), and accumulating evidence suggests that cross talk between these hormones, particularly at the level of their central intracellular signal transduction, leads to synergistic action in the regulation of energy balance (11, 30, 41).

The insulin receptor (IR) is a tetrameric complex composed of two α- and β-subunits. Although not related to the leptin receptor, the IR shares the feature of possessing intrinsic tyrosine-protein-kinase activity leading to activation of downstream signaling pathways (39). Cellular interaction of leptin and insulin signaling is most likely to occur via a pathway involving phosphatidylinositol 3-kinase (PI3-kinase) (28, 31, 41). Upon insulin binding, PI3-kinase is activated by IR substrate proteins that have been phosphorylated by the IR (16, 27). PI3-kinase catalyzes the phosphorylation of phosphatidylinositol 4,5-bisphosphate to phosphatidylinositol 3,4,5-trisphosphate, which, in turn, activates downstream targets like AKT (also known as protein kinase B), a pivotal molecule for most of insulin’s effects (14, 30).

Inhibitory molecules, such as the suppressor of cytokine signaling 3 (SOCS3) and several protein tyrosine phosphatases ([PTPs] PTPα, LAR, CD45, PTPε, and PTP1B), are thought to deactivate insulin signaling, whereas some of them (SOCS3 and PTP1B) even exhibit synergistic effects in terms of inhibition of both leptin and IR signaling (2, 26). Among these molecules, substantial evidence supports PTP1B as being the central player in controlling insulin action. PTP1B knockout in mice and knockout by antisense oligonucleotides in diabetic rodents leads to enhanced insulin sensitivity (10, 17, 36, 46). These animals maintain euglycemia (in the fed state) with one-half the level of insulin observed in wild-type littersmates and, surprisingly, are resistant to diet-induced obesity. These attributes make this phosphatase a very attractive candidate for obesity and type 2 diabetes research.

Our knowledge of insulin signaling pathways and the molecules involved is derived from studies investigating insulin’s action in the periphery. The only study investigating the
peripheral effect of insulin on seasonal body weight regulation in *P. sungorus* was compromised by the adverse response to streptozotocin treatment (5). Although recent studies reported blockade of intracerebroventricular (ICV) insulin actions on both food intake and hepatic glucose production by ICV pretreatment with PI3-kinase inhibitors (28, 31), the details of central insulin signal transduction remain limited.

Recent studies have unmasked a seasonal cycle in sensitivity to the adipocyte-derived hormone leptin in *P. sungorus* (3, 19, 37). We proposed that SOCS3 plays an important role in mediating seasonal modifications in leptin sensitivity, suggesting that the underlying molecular mechanism is centered on intracellular signal transduction of leptin receptors in the arcuate nucleus, the brain region with strongest density of SOCS3 gene expression (43).

The close association of leptin and insulin signaling raises the question of whether modifications in insulin signal transduction within the arcuate nucleus are implicated in seasonal body weight regulation. In the present study, we investigated this idea by analyzing (in situ hybridization) hypothalamic IR, PTP1B, and PI3-kinase gene expression in juvenile female hamsters that had been acclimated to either SD or LD for a period of 8 wk. Furthermore, we investigated phosphorylation of AKT in the hypothalamus, detected with phosphospecific antibodies by immunoblotting and immunohistochemistry, and determined the effect of photoperiod on the hypothalamic content of this pivotal insulin signaling molecule.

**MATERIALS AND METHODS**

All procedures involving animals were licensed under the Animals (Scientific Procedures) Act of 1986 and received approval from the Ethical Review Committee at the Rowett Research Institute. Siberian hamsters were drawn from the Rowett breeding colony (1, 21, 23) and were gestated and suckled in a LD photoperiod (16:8-h light-dark cycle). All Siberian hamsters were weaned at 3 wk of age and were individually housed at weaning. Where specified, hamsters were transferred into a SD photoperiod (8:16-h light-dark cycle). All Siberian hamsters were weaned at 3 wk of age and were additionally housed in LD for the same period. For all experiments, juvenile female hamsters (weaned at 3 wk of age) or transferred to SD photoperiod for the same period (n = 19) or transferred to SD photoperiod for the same period (n = 19) postweaning. For analysis of IR, PTP1B, and PI3-kinase gene expression by in situ hybridization in either photoperiod, six food-deprived (48 h) and six ad libitum-fed control animals were killed. For detection of phospho-AKT by immunoblotting or immunohistochemistry, respectively, three or four brains from ad libitum-fed hamsters from each photoperiod were used. PTP1B mRNA in ad libitum-fed animals was analyzed in archived brain sections from an earlier experiment performed under identical conditions (43).

**Hypothalamic Gene Expression**

mRNA levels were quantified by in situ hybridization in 20-μm coronal hypothalamic sections by using techniques described in detail elsewhere (23). Riboprobes complementary to IR, PI3-kinase, and PTP1B were generated from cloned cDNA from the hypothalamus of the Siberian hamster. cDNA synthesis was performed by using a cDNA synthesis kit (Invitrogen, Carlsbad, CA), according to the manufacturer’s instructions. Primers for amplification of the three fragments were designed using Primer Select (Table 1; Lasergene, DNA-Star Software). The IR amplicons were generated by PCR with 35 cycles of 94°C for 1 min, 55°C for 1 min 40 s, and 72°C for 2 min, and a final extension at 72°C for 10 min. For the amplification of PI3-kinase and PTP1B, the annealing temperature was adjusted to 50°C for PI3-kinase and 59°C for PTP1B. DNA fragments were ligated into pGEM-T-Easy [(IR and PI3-kinase), Promega, Madison, WI] or pPCR-Script Amp SK+ [(PTP1B) Stratagene, La Jolla, CA] transformed into *Escherichia coli* DH5α and sequenced. For cRNA synthesis of antisense riboprobes by in vitro transcription SP6-polymerase (IR and PI3-kinase; Invitrogen) or T3-polymerase (PTP1B; Invitrogen) were used. To generate the sense control for all three riboprobes, cRNA synthesis was performed by T7-polymerase.

As previously described (23), forebrain sections (20 μm) were collected throughout the extent of the arcuate nucleus (ARC) onto a set of eight slides with six or seven sections mounted on each slide. Accordingly, slides spanned the hypothalamic region approximating from −2.7 to −1.46 mm relative to Bregma according to the atlas of the mouse brain (33). One slide from each animal was hybridized. Briefly, slides were fixed, acetylated, and hybridized overnight at 58°C using 35S-labeled cRNA probes (1–2 × 10⁶ cpm/ml). Slides were treated with RNase A, desalted with a final high-stringency wash (30 min) in 0.1 × SSC at 60°C, dried, and exposed to Kodak Biomax MR Film (Kodak, Rochester, New York, NY). Autoradiographic images were quantified using the Image-Pro Plus system. Corresponding sections of individual animals were matched according to the atlas of the mouse brain. Three to four sections from the ARC of each animal spanning from −2.54 to −1.94 mm relative to Bregma were

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**Table 1. Oligonucleotides used for cloning of the respective candidate genes for in situ hybridization**

<table>
<thead>
<tr>
<th>Probes</th>
<th>Primers</th>
<th>Oligonucleotide Sequence</th>
<th>Fragment Size, bp</th>
<th>GenBank Accession No., rat/mouse</th>
</tr>
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<tr>
<td>Insulin receptor</td>
<td>Forward</td>
<td>5’-CTGGGGCCGGAGTTGGAAG-3’</td>
<td>229</td>
<td>8393620</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-CCCTGCAGCCGCCTTCCAGTAA-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI3-Kinase</td>
<td>Forward</td>
<td>5’-AACGGAAGGCAGAGAAGAGA-3’</td>
<td>219</td>
<td>6981357</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-AACGGAAGGCAGAGAAGAGA-3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTP1B</td>
<td>Forward</td>
<td>5’-TGCCACAACGAGAAGAGAGAG-3’</td>
<td>409</td>
<td>50872126</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-TGACGCCGACTTGCGGAAAG-3’</td>
<td></td>
<td></td>
</tr>
</tbody>
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PI3-kinase, phosphatidylinositol 3-kinase; PTP1B, protein tyrosine phosphatase 1B.

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analyzed. Data were manipulated using a standard curve generated from $^{14}$C autoradiographic microscales (Amersham Pharmacia Biotech), and the integrated intensities of the hybridization signals were computed.

**Immunohistochemistry**

For immunohistochemistry, animals were anesthetized with Euthatal (Rhone Merieux, Harlow, UK), transcardially perfused with 0.9% saline containing heparin (1,000 U/l), and followed by 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed and postfixed in the same fixative overnight at 4°C. On the next morning, brains were transferred to 30% sucrose in 0.1 M phosphate buffer for dehydration and cryoprotection. When brains had sunk, they were frozen in isopentane, cooled on dry ice for 1 min, and sectioned coronally at 35 µm throughout the extent of the hypothalamus (additionally the nucleus tractus solitarius region of the hindbrain was cut) on a freezing microtome, collected in four series, and stored in cryoprotectant at 4°C. Free-floating sections were incubated in 1% H$_2$O$_2$, 10% methanol diluted in H$_2$O for 15 min to quench endogenous peroxide followed by incubation in blocking solution (5% BSA, 0.5% Triton X-100 in phosphate buffer) for 45 min. Sections were incubated overnight at 4°C with anti-phospho-AKT primary antibody (Ser473, IHC-specific; cat. no. 9277, Cell Signaling Technology) diluted in blocking solution (1:100). On the next day, sections were incubated with a biotinylated secondary goat anti-rabbit antibody for 1 h (1:1,000, in blocking solution containing 3% BSA), and then treated with ABC (Vector Laboratories, Burlingame, CA) solution for 2 h. Between steps, sections were washed in phosphate buffer containing 0.25% Triton X-100. Finally, the signal was developed with nickel-DAB solution (Vector Laboratories), giving a gray/black precipitate. Section images were captured by using a Polaroid DMCe digital camera mounted on a Zeiss Axioskop (Jena, Germany).

**Immunoblotting**

For immunoblotting, hypothalami were immediately excised with anatomical precision from freshly prepared brains, weighed, and rapidly frozen in liquid nitrogen. With the use of a glass homogenizer, tissues were homogenized in buffer containing phosphatase and protease inhibitors (in mM: 10 HEPES pH 7.9, 1.5 MgCl$_2$, 10 KCl, 0.5 DTT, 0.5 PMSF, 20 NaF, and 1 Na$_3$VO$_4$) and incubated on ice for 10 min. For detection of phospho-AKT, the cytoplasmic fraction was separated from the nuclear part by centrifugation for 15 min at 3,300 g. The protein content of the supernatant containing the cytoplasmic fraction was determined by the Bradford assay, and equal amounts of protein were loaded into each lane. Immunoblotting analysis was performed by standard method (18). Samples were separated on 12.5% SDS-PAGE and, after transfer to nitrocellulose membrane, stained with red Ponceau dye to ensure accurate protein loading and transfer. Phospho-AKT was detected by immunodetection. The extent of the hypothalamus (additionally the nucleus tractus solitarius region of the hindbrain was cut) on a freezing microtome, collected in four series, and stored in cryoprotectant at 4°C. Free-floating sections were incubated in 1% H$_2$O$_2$, 10% methanol diluted in H$_2$O for 15 min to quench endogenous peroxide followed by incubation in blocking solution (5% BSA, 0.5% Triton X-100 in phosphate buffer) for 45 min. Sections were incubated overnight at 4°C with anti-phospho-AKT primary antibody (Ser473, IHC-specific; cat. no. 9277, Cell Signaling Technology) diluted in blocking solution (1:100). On the next day, sections were incubated with a biotinylated secondary goat anti-rabbit antibody for 1 h (1:1,000, in blocking solution containing 3% BSA), and then treated with ABC (Vector Laboratories, Burlingame, CA) solution for 2 h. Between steps, sections were washed in phosphate buffer containing 0.25% Triton X-100. Finally, the signal was developed with nickel-DAB solution (Vector Laboratories), giving a gray/black precipitate. Section images were captured by using a Polaroid DMCe digital camera mounted on a Zeiss Axioskop (Jena, Germany).

**Statistical Analysis**

Data were analyzed by one- or two-way ANOVA followed by Student-Newman-Keuls multiple comparison test, as appropriate, using SigmaStat statistical software (Jandel, Erkrath, Germany). Where data failed equal variance or normality tests they were analyzed by one-way ANOVA on ranks followed by Dunn’s multiple comparison test. Results are presented as means ± SE, and differences were considered significant if $P < 0.05$.

**RESULTS**

**Distribution of IR, PI3-Kinase, and PTP1B Gene Expression in the Hamster Brain**

The species-specific probes to IR, PI3-kinase, and PTP1B mRNA had an identity of 95, 91, and 90% in nucleic acid sequence to *Rattus norvegicus* or *Mus musculus*, respectively (for GenBank accession nos., see Table 1). Within the investigated brain region, neuroanatomical structures that hybridized the three riboprobes are listed in Table 2 along with their estimated relative intensities (see also, Fig. 1). Of particular interest were the localized and intense hybridization signals of IR, PI3-kinase, and PTP1B in the ARC. PI3-kinase gene expression in the thalamus was relatively uniform and could not be attributed to individual thalamic nuclei. For all three candidate genes, sense probes synthesized from the cloned cDNA generated a low-intensity nonspecific signal (Fig. 1D).

**Effect of Photoperiod and/or Food Deprivation on Insulin Signaling Components**

**Effect of photoperiod and food deprivation on IR gene expression.** Body weight change at 8 wk postweaning was similar to an identical experiment reported earlier (43). Over the 8-wk period, SD hamsters gained 10.2 ± 1.3 g, while hamsters in LD gained 14.2 ± 1.2 g. Uterine tract size was reduced in SD acclimated hamsters compared with controls. In juvenile female hamsters acclimated to SD photoperiod for 8 wk, IR gene expression was decreased significantly compared with hamsters maintained in LD for the same period (two-way ANOVA; $F = 5.909$; $P < 0.05$; Fig. 1A). Although there was a trend to decreased IR gene expression in response to food deprivation in LD, this difference was not significant, and there was no significant interaction between photoperiod and food deprivation. IR gene expression in structures analyzed outside the ARC was unaffected by either photoperiod or feeding status. SD-induced downregulation of IR to about 40% of the expression level in LD hamsters was confirmed by repeating the experiment with 11–12 ad libitum-fed female hamsters in each photoperiod [8 wk postweaning, (one-way ANOVA on ranks; $H = 8.37$; $P < 0.001$; data not shown)].

**Effect of photoperiod and food deprivation on PI3-kinase gene expression.** There was no effect of photoperiod, feeding status, or interaction of both parameters on PI3-kinase mRNA expression.

| Table 2. Distribution of mRNA in the investigated brain region |
|------------------|------------------|------------------|
| **Structure**    | **IR**           | **PI3-Kinase**   | **PTP1B**      |
| CA1-3            | ++ +             | ++ +             | ++ +           |
| dentate gyrus    | ++ +             | ++ +             | ++ +           |
| habenular nucleus| ++ +             | ++ +             | ++ +           |
| choroid plexus   | ++ +             | ++ +             | ++ +           |
| cerebral cortex  | ++ +             | ++ +             | ++ +           |
| piriform cortex  | ++ +             | ++ +             | ++ +           |
| amygdala         | ++ +             | ++ +             | ++ +           |
| optic tract      | ++ +             | ++ +             | ++ +           |
| accum nucleus    | ++ +             | ++ +             | ++ +           |
| thalamus         | ++ +             | ++ +             | ++ +           |

LD, long-day line; IR, insulin receptor.
in any of the brain regions examined (Fig. 1B), although the latter almost achieved significance with \( P = 0.061 \).

**Effect of photoperiod and food deprivation on PTP1B gene expression.** A highly significant reduction of PTP1B mRNA levels in the ARC was observed (Fig. 1C) in response to SD acclimation (two-way ANOVA, \( F = 52.24; P < 0.001 \)) and to food deprivation (two-way ANOVA, \( F = 7.74; P < 0.05 \)). Additionally, there was a significant interaction between photoperiod and feeding status (two-way ANOVA, \( F = 7.14; P < 0.05 \)). Multiple group-wise comparisons revealed a sig-
significant reduction of ARC PTP1B gene expression induced by food deprivation in LD but not in SD (Fig. 1C). There was also a highly significant increase in PTP1B gene expression in the thalamus in response to food deprivation (two-way ANOVA, $F = 39.73; P < 0.0001$) but not in response to photoperiod (Fig. 1C and Fig. 2). PTP1B gene expression was unaffected by photoperiod or feeding status in other analyzed structures.

Effect of photoperiod on the phosphorylation of AKT. As a marker for PI3-kinase activity we determined the hypothalamic content of phosphorylated AKT. As shown in Fig. 3A, a single conspicuous and specific band of the expected size (60 kDa) for phospho-AKT could be determined in hypothalamic lysates of the Siberian hamster. Exposure of juvenile female hamsters to SD for 8 wk led to a striking reduction of phospho-AKT in the hypothalamus compared with LD hamsters as revealed by quantification of the immunoblot (Fig. 3B).

With the use of immunohistochemistry, phospho-AKT positive cells were detected in the ARC. The very specific signal was conspicuously confined to this brain region (Fig. 3C) and appeared to be located within the entire cell (Fig. 3D). Beyond the ARC, phospho-AKT immunoreactive cells were only present in the nucleus tractus solitarius of the hindbrain (data not shown). The differences revealed by immunoblotting were investigated at a neuroanatomical level by immunohistochemistry. As mentioned above, phospho-AKT immunoreactivity was restricted to the ARC and photoperiod-induced differences were confined to this region (Fig. 3C). Thus the changes observed by immunoblotting of total lysates were due to localized changes in the ARC. Consistently, in all four analyzed animals from each photoperiod, phospho-AKT immunoreactivity was substantially lower in SD compared with LD.

DISCUSSION

Over the last 5 years, accumulating evidence has revealed a seasonal alteration in central leptin signaling in P. sungorus reflected by increased leptin sensitivity in SD and the establishment of central leptin resistance in LD (3, 19, 37, 43). Leptin exhibits conspicuous similarities in its central effects with insulin, the second hormone besides leptin that meets the criteria for an “adiposity signal”. Growing evidence in the literature suggests convergence of central leptin and insulin signaling at the level downstream of their respective receptors.
The present study was designed to investigate seasonal alterations in central signal transduction of insulin. Here we report downregulation of central IR gene expression in response to SD in the seasonal hamster *P. sungorus*. This was associated with a marked reduction of hypothalamic phospho-AKT either due to reduced phosphorylation of the total AKT pool or a chronic decline of total AKT protein abundance. These changes were accompanied by diminished gene expression of PTP1B, the main inhibitor of insulin signaling.

The neuroanatomical gene expression pattern of IR and PI3-kinase is consistent with those reported in rats and mice, respectively (13, 20). Expression was mainly confined to the ARC, a key neuronal center for the integrated regulation of body weight. This together with the fact that IR gene expression was profoundly downregulated by SD photoperiod in this area indicates that insulin signaling may be involved in the seasonal body weight cycle exhibited by *P. sungorus*.

Seasonally induced alterations in circulating insulin levels were reported in the related species *P. campbelli* (22). In adult male hamsters after a 20-wk acclimation, SD plasma insulin levels fell to about 20% of the level in LD controls (22).

In *P. sungorus*, presumed elevation of insulin levels in LD coincident with increased IR gene expression in the ARC suggests a causal link. Entry of insulin into specific brain areas across the blood-brain barrier is well established (4, 39). Although regional uptake of insulin into the brain does not directly correlate with the localization of the IR (the pons-medulla is the region with the greatest insulin passage Ki:0.764 μg/min, but it only contains relatively few insulin IRs, (4)), intriguingly, the second largest flux of circulating insulin into the brain is located within the hypothalamus (Ki:0.731 μg/min), substantiating the importance of regional density of IR molecules in this area. Although it is well documented that brain insulin is probably largely of peripheral origin, the possibility of local insulin synthesis cannot be excluded and remains controversial. It has been reported that the concentrations of insulin in the cerebrospinal fluid and the entry of insulin into the hypothalamus positively correlate with feeding status, which may provide a link between the seasonal alterations in IR gene expression and the marked seasonal body weight cycle that is reflected in food intake (12, 32, 40). An important association of brain IR and feeding status is also substantiated by the trend to decreased IR gene expression in the ARC in LD hamsters in response to food deprivation (48 h). This effect could not be observed in SD, suggesting that these animals exhibit a basal IR gene expression level.

Considering the potent anorexigenic action of central insulin, SD-induced downregulation of circulating insulin signaling in the brain appears to be paradoxical. Recent studies demonstrated that third cerebral ventricle (ICV) administration of insulin decreases food intake and body weight (35, 41, 44, 45). Furthermore, diminished insulin signaling, as it was achieved by neuronal knockout, or knockdown of the IR (by administration of antisense oligonucleotides against the IR) or neuronal-specific reduction of insulin signaling distal to its receptor (by ICV treatment with PI3-kinase inhibitors), led to hyperphagia and an increase in body weight (7, 29, 31). In Siberian hamsters, however, diminished hypothalamic signal transduction of this hormone (as exemplified by reduction of IR mRNA and phospho-AKT protein) occurs in SD, at times when body weight is low and food intake is comparatively reduced. The finding that both IR gene expression and insulin signaling via phospho-AKT are downregulated in SD hamsters contrasts to studies undertaken in other rodent species subjected to extreme genetical or pharmacological modifications. Clearly, *P. sungorus* reveals adaptive physiological responses, and seasonal cycles in body weight may not exclusively rely on the fundamental catabolic action of insulin described in the other studies mentioned above. Conceivably, reduced insulin signaling in SD may be the result rather than the trigger of an increased catabolic tone mediated by enhanced leptin sensitivity in this photoperiod. Reduced central insulin signaling possibly prevents a catabolic overdrive which would have resulted from additive actions of both hormones. This hypothetical scenario may be critical for the survival of Siberian hamsters in harsh winter conditions, times when food is limited. Nevertheless, our study supports central insulin signaling mediated via PI3-kinase since both IR and phospho-AKT were consistently coregulated in response to photoperiod within the ARC.

Beside the PI3-kinase pathway, other pathways (JAK-STAT and ERK) (8, 9) have been implicated in peripheral insulin signaling. Convergence of both insulin and leptin signal transduction upon the level of these other pathways beyond signaling through PI3-kinase is plausible.

The role of PTP1B as a central player in modulating insulin signal transduction is intriguing, but may be challenged by the fact that gene expression of this inhibitory molecule is reduced in SD when insulin signaling is minimal. Assuming that the reduction of PTP1B mRNA is reflected at the protein level, lowered content of this inhibitor in the ARC would be expected to result in increased insulin signaling. Nevertheless, the hypothalamic phospho-AKT content was substantially reduced in SD when insulin signaling is minimal. Assuming that the reduction of PTP1B mRNA is reflected at the protein level, lowered content of this inhibitor in the ARC would be expected to result in increased insulin signaling. Nevertheless, the hypothalamic phospho-AKT content was substantially reduced in this photoperiod. We did not investigate functional interactions of the insulin signaling components. Although the regulation of the expression levels in response to SD and food deprivation for 48 h of both IR and PTP1B are almost identical, indicating strong interaction, it is possible that changes in brain PTP1B gene expression may not be exclusively associated with insulin signaling. The intriguing finding that PTP1B gene expression is massively induced by food deprivation in the thalamus, a region that in Siberian hamsters does not express either IR or the long form of the leptin receptor (with full signal transduction capacity), indicates that PTP1B may be involved in signaling of other feeding-status regulatory hormones whose identities are presently unknown. Interestingly, Purvis et al. (34) demonstrated that neurotoxic lesions of the midline thalamus (reunions nucleus) inhibited SD-induced loss of body weight in *P. sungorus*. This implies a possible regulatory role of PTP1B in mediating seasonal changes in body weight within this neuroanatomical structure.

PTP1B has also been associated with intracellular signaling of the second “adiposity signal,” leptin. This factor exhibits its inhibitory effects on leptin signaling most likely via deactivation of the Janus kinase 2, an enzyme associated with the leptin receptor (15). Seasonal modulation of leptin signaling by PTP1B may be plausible. Supporting evidence for this hypothesis is contributed by the identical neuroanatomical expression patterns of both PTP1B and leptin receptor mRNA within the ARC of the Siberian hamster (24). Moreover, the mRNA of SOCS3, a possible key modulator in the mediation of the seasonal cycle in leptin sensitivity, reveals a very similar hypothalamic neuroanatomical expression pattern like PTP1B.
mRNA and in response to SD ARC, SOCS3, and PTP1B gene expression are diminished to a strikingly similar extent (43). PTP1B and SOCS3 may exhibit potent synergistic actions in terms of inhibition of insulin and leptin signaling. The complementary deactivatory attributes of both molecules are unlikely to be primarily displayed on the level of the PI3-kinase (due to the lack of increased PI3-kinase activity in SD); it is rather plausible that intracellular signaling is modified by additional recruitment of the JAK-STAT or ERK signaling pathways. Diminished inhibition of leptin signaling in SD on the level of the JAK-STAT pathway is also supported by the very recent finding of a substantial increase of phospho-STAT3 positive neurons within the hypothalamus in response to a single intraperitoneal leptin injection (Tups A, unpublished observations). These data substantiate the close association of leptin and insulin signaling within the hypothalamus. The decline of insulin signaling in SD may be primarily the response to the reduced fat mass and the metabolic modifications in SD. The apparent paradoxically reduced anorexigenic drive of insulin implicated by marked diminished insulin signaling may be antagonized by increased activation of the JAK-STAT pathway induced by enhanced leptin sensitivity in SD. This concept is corroborated by a very recent study performed by Sahu and Metlakunta (38) who showed that, in rats under chronic central leptin infusion for 16 days, the JAK-STAT pathway remains sensitive to exogenous leptin administration, whereas, under these circumstances, leptin treatment fails to induce PI3-kinase activity. Furthermore, in a human neuronal cell line, Benomar et al. (6) demonstrated that indeed both insulin and leptin are able to induce the JAK-STAT pathway and coadministration of both hormones further elevated STAT3 phosphorylation. In contrast, PI3-kinase signaling, despite being induced by both hormones individually, is not further enhanced by the presence of both insulin and leptin. Thus convergence of both insulin and leptin signaling within the hypothalamus and partial redundancy of their signal transduction cascades may be a central requirement for the dynamic regulation of energy homeostasis.

In summary, we provide evidence that seasonal body weight regulation is associated with modulations in ARC insulin signal transduction. The direction of change in neuronal insulin signaling, however, contrasts to the central catabolic nature of this pathway described in other species. Reduced insulin signaling in SD may result from enhanced leptin signaling in SD as a consequence of reduced body fat stores. IRSs and leptin receptors are both expressed in the ARC of Siberian hamsters but intracellular signaling of both hormones is inverse, making it unlikely that both hormones exhibit synergistic body weight regulatory effects in the hypothalamus. We cannot, however, rule out possible inverse cross talk of both hormones. Beyond PI3-kinase, insulin and leptin share further signal transduction pathways. This cross talk of insulin and leptin within the hypothalamus, distal of their respective receptors, remains an enigma whose resolution will certainly enable us to better understand the complex mechanisms maintaining energy homeostasis and that may be perturbed in obesity.

REFERENCES

INSULIN SIGNALING IN ARCUATE NUCLEUS OF SIBERIAN HAMSTER


