Differential Acute and Chronic Effects of Leptin on Hypothalamic Astrocyte Morphology and Synaptic Protein Levels

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Astrocytes participate in neuroendocrine functions partially through modulation of synaptic input density in the hypothalamus. Indeed, glial ensheathing of neurons is modified by specific hormones, thus determining the availability of neuronal membrane space for synaptic inputs, with the loss of this plasticity possibly being involved in pathological processes. Leptin modulates synaptic inputs in the hypothalamus, but whether astrocytes participate in this action is unknown. Here we report that astrocyte structural proteins, such as glial fibrillary acidic protein (GFAP) and vimentin, are induced and astrocyte morphology modified by chronic leptin administration (intracerebroventricular, 2 wk), with these changes being inversely related to modifications in synaptic protein densities. Similar changes in glial structural proteins were observed in adult male rats that had increased body weight and circulating leptin levels due to neonatal overnutrition (overnutrition: four pups/litter vs. control: 12 pups/litter). However, acute leptin treatment reduced hypothalamic GFAP levels and induced synaptic protein levels 1 h after administration, with no effect on vimentin. In primary hypothalamic astrocyte cultures leptin also reduced GFAP levels at 1 h, with an induction at 24 h, indicating a possible direct effect of leptin. Hence, one mechanism by which leptin may affect metabolism is by modifying hypothalamic astrocyte morphology, which in turn could alter synaptic inputs to hypothalamic neurons. Furthermore, the responses to acute and chronic leptin exposure are inverse, raising the possibility that increased glial activation in response to chronic leptin exposure could be involved in central leptin resistance. (Endocrinology 152: 1809–1818, 2011)

The increasing incidence of obesity continues to be a major medical concern in the developed world, despite the fact that much progress has been made in our understanding of the control of appetite and metabolism. Indeed, it is now widely accepted that not only are there genetic influences on our propensity to become overweight but also that early life experiences, including nutrition, also play a fundamental role in this process (1–4).

Some of these early events induce structural changes in brain areas involved in metabolic control, including the hypothalamus, modulating its response to metabolic signals during adulthood and increasing the probability of excess weight gain. For example, the density of synaptic inputs to hypothalamic nuclei and specific neuronal populations involved in metabolic control are altered in response to modifications in leptin signaling or increased...
body weight (bw) during early development (3, 5–7). Indeed, leptin affects neurite outgrowth as well as both neuronal and glial development and changes in this hormone during brain development clearly affect later metabolic responses (3, 6–8). Furthermore, hypothalamic neuronal circuits are rapidly modulated by leptin in the adult animal (5). Thus, not only does leptin induce changes in neuropeptide synthesis and secretion (9), but it also modulates the development and structure of neuronal circuits involved in metabolic control.

Astrocytes actively participate in developmental processes, maintenance of the local neuronal environment, synaptic remodeling and transmission, transport of substances from the periphery, and glucose metabolism (10, 11). These glial cells are also intimately involved in central inflammatory responses, with the role of hypothalamic inflammation in the control of energy balance currently receiving much attention (12). Moreover, hypothalamic astrocytes participate in the synaptic rearrangement involved in neuroendocrine functions and in response to specific hormones such as estrogens (13–16). These glial cells extend their projections and coverage of neurons in response to specific signals, and this can be inversely correlated with the number of synaptic inputs, thus modifying the responsiveness of specific hypothalamic circuits. However, the role of astrocytes in the control of metabolic functions has only recently been addressed (17–19). Hypothalamic astrocytes have been reported to express leptin receptors (17, 18, 20), although expression of functional forms of this receptor is still conjectural. Because the astrocyte structural filament glial fibrillary acidic protein (GFAP) is increased in models of obesity (17, 19) and leptin modifies synaptic inputs in the hypothalamus (5), it is possible that glial cells participate in this leptin-induced synaptic rearrangement.

Here we addressed the question as to whether overnutrition during early development, which results in increased body weight during adulthood, modifies hypothalamic glial structural proteins and whether this is associated with changes in synaptic proteins. Furthermore, acute and chronic leptin treatments, both in vivo and in vitro, were used to determine the short- and long-term effects of this hormone on hypothalamic astrocytes and the possible role that these glial cells may play in leptin-induced synaptic rearrangement.

**Materials and Methods**

All studies were approved by the local ethics committee and complied with the Royal Decree 1201/2005 (Boletín Oficial del Estado, BOE no. 252) pertaining to the protection of experimental animals and with the European Communities Council Directive (86/609/EEC).

**Adjustment of litter size**

On the day of birth, litters of Wistar rats were adjusted to small litters of two males and two females (SmL, n = 6) or control litters of six males and six females per dam (Ctl., n = 8). Only males from at least two different litters were used in these studies. After weaning on postnatal d 21, rats were placed two to a cage and allowed free access to rat chow and water. Food intake and bw were determined twice weekly until the rats were killed by decapitation at postnatal d 70 between 0900 and 1100 h.

**Chronic leptin treatment**

Adult male Wistar rats (250–300 g; n = 6/group) were anesthetized with 2.5% Fluotane (AstraZeneca Farmacéutica S.A, Madrid, Spain) and a minipump (Alzet; Durect Co., Cupertino, CA) that delivered either 15 μg/d leptin (0.625 μg/h; National Hormone and Pituitary Program, Torrance, CA) or saline for 14 d was connected to a cannula that was implanted in the left lateral ventricle (−0.3 mm anteroposterior, 1.1 mm lateral from bregma). Rats were weighed every 2 d until they were killed between 0900 and 1100 h by decapitation.

**Acute leptin treatment**

Twenty-eight adult male Wistar rats (250 ± 10 g) were anesthetized (0.02 ml ketamine per 100 g bw and 0.04 ml xylazine per 100 g bw) and a cannula attached to a catheter implanted in the left lateral cerebral ventricle (−0.3 mm anteroposterior, 1.1 mm lateral from bregma). Two days after surgery, including a fasting period of 12 h, rats received either 3 μg leptin dissolved in 7 μl saline or 7 μl of vehicle at 1000 h. Rats were killed by decapitation immediately after receiving vehicle (defined as 0 h) or at 1 or 6 h after injection of either vehicle or leptin. In all in vivo studies the brains were quickly removed and the hypothalamus and cerebellum dissected on dry ice and immediately stored at −80 C. Trunk blood was collected and the serum collected and stored at −80 C.

**Primary astrocyte cultures**

The diencephalon was removed from 2-d-old male Wistar rats and triturated in DMEM F-12 (Life Technologies, Inc., Invitrogen Corp., Carlsbad, CA) containing 1% penicillin-streptomycin. The suspension was centrifuged for 7 min at 1000 rpm and the pellet resuspended in DMEM F-12 + 10% fetal calf serum (FCS; Linus, Madrid, Spain) + 1% antibiotics. One milliliter of cell suspension was added to 9 ml culture media in a 75-cm² culture flask and incubated at 37 C and 5% CO₂ for 9 d, changing the media every 3 d. The flasks were then placed in a 37 C shaking incubator at 280 rpm for 16 h. The cells were washed, incubated for 2 min at 37 C with a 0.05% trypsin/EDTA solution (Biochrom AG, Berlin, Germany), resuspended in DMEM F-12 + 10% FCS + 1% antibiotics, and centrifuged for 5 min at 1150 rpm. The pellet was resuspended in the same media and seeded at a concentration of 217,500 cells/ml in culture plates treated poly-L-lysine hydrobromide (10 μg/ml; Sigma, St. Louis, MO). Twenty-four hours later the media were changed to DMEM F-12 + 1% antibiotics (without FCS). For analysis of protein levels, the media were removed 24 h later and fresh DMEM F-12 + 1% antibiotics plus saline or 100 ng/ml of recombinant rat leptin added during 1, 6, or 24 h. Each treatment was done in triplicate in each experiment, and each experiment was repeated seven times. For analysis of leptin receptor mRNA levels, astrocyte
cultures were incubated with 0, 10, or 100 ng of leptin for 24 h. This experiment was repeated three times.

This procedure resulted in a purity of greater than 95% astrocytes.

Leptin, insulin, and adiponectin ELISAs

These hormones were measured by ELISA following the manufacturer’s instructions (Linco Research Inc., St. Charles, MO). The sensitivity of the assays for leptin, insulin, and adiponectin were 0.04, 0.2, and 0.16 ng/ml, respectively. The intra-assay variation was 2.2% for leptin, 1.9% for insulin, and 1.3% for adiponectin, and the interassay variations were 3.4% for leptin, 7.6% for insulin, and 7.0% for adiponectin. All samples were run in duplicate.

Western blotting

Hypothalami and cerebellar cortex were homogenized in radioimmunoprecipitation assay lysis buffer as previously described (21). For in vitro experiments, lysis buffer containing 25 mM HEPES, 150 mM KCl, 2 mM of EDTA, 0.1% Igepal, 1 mM phenylmethylsulfonyl fluoride, 10 μM benzamidine and leupeptin, and 0.5 mM dithiothreitol was added to the culture plates. Total protein concentrations were determined by the method of Bradford (Bio-Rad Laboratories, Hercules, CA).

Depending on the protein to be detected, 10, 20, or 60 μg of protein was resolved on an 8–12% sodium dodecyl sulfate-polyacrylamide gel under denaturing conditions, electrotransferred to polyvinyl difluoride membranes (Bio-Rad), and blocked in Tris-buffered saline (20 mM) containing 5% nonfat dried milk to polyvinyl difluoride membranes (Bio-Rad, Hercules, CA). All primary antibodies were used at a dilution of 1:1000 and incubated overnight at 4°C under agitation. The antibody conjugated with peroxidase (Pierce, Rockford, IL), and/or GAPDH levels in each lane and normalized to Ponceau and/or GAPDH levels in each lane and normalized to the optical disector technique (23). The number of primary projections was determined for each GFAP positive cell that was included entirely in the field of analysis and Sholl's analysis (24) was performed to assess differences in the extension of glial processes as described by Del Cerro et al. (25).

Briefly, an overlay of 10 concentric rings centered on the soma of each astrocyte and all intersections of the GFAP-immunoreactive processes with the graticule were counted. The separation between the annuli of the graticule was equivalent to 3.57 μm.

For analysis of the number of vimentin labeled fibers, five sections distributed throughout the arcuate nucleus were analyzed in each animal. In each tissue section, 10–15 images corresponding to an area of 100 μm × 100 μm were captured. Images were randomly taken along the third ventricle but excluded the layer of cell bodies surrounding the ventricle. Images were captured using a digital camera and a ×40 objective and processed using Image-Pro Plus software (Media Cybernetics). All vimentin labeled fibers running perpendicular in respect to the third ventricle were counted.

All analyses were performed without previous knowledge of the experimental group being analyzed. The error of repetition of the operator was less than 5%.

RNA preparation and purification and semiquantitative real-time PCR

Total RNA was extracted according to the Tri-Reagent protocol from primary hypothalamic astrocyte cultures incubated with 0, 10, or 100 ng of leptin for 24 h. cDNA was synthesized from 1 μg of total RNA by using a high-capacity cDNA reverse transcriptase kit (Applied Biosystems, Foster City, CA). Semi-quantitative real-time PCR was performed using an assay-on-demand kit (Applied Biosystems) for ObR (Rn01433205 Hs, Cq = 0.19, Rn01433206 Hs, Cq = 0.11), and TaqMan universal PCR master mix (Applied Biosystems) according to the manufacturer’s protocol in an ABI PRISM 7000 sequence detection system (Applied Biosystems). Values were normalized to the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase. According to manufacturer’s guidelines, the ΔΔCt method (comparative CT method, or 2-ΔΔCt method where ΔΔCt = ΔCt, sample − ΔCt, reference) was used to determine relative expression levels.

Statistical analysis

A two-tailed student’s t test was used for comparison between groups when only two groups were analyzed. Changes in weight over time were analyzed by repeated measures. A two-way ANOVA was used to determine the effect of leptin over time. A one-way ANOVA followed by a Scheffé-F test was then used.
when indicated. In vivo experiments were performed a minimum of two times. In vitro experiments were performed either three or seven times with three replicates of each treatment in each experiment (n = 3 or 7). Statistical significance was chosen as P < 0.05. All results are reported as mean ± SEM.

Results

Effect of litter size

At weaning, pups from small litters (four pups) weighed more than those from litters of 12 (Ctl: 34.5 ± 0.4 g; SmL: 48.8 ± 1.2 g; P < 0.001) and continued to do so throughout the study, although this difference was not significant again until the seventh week after weaning, or 10 wk of age (Ctl: 292.8 ± 8.9 g; SmL: 247.9 ± 7.3 g; P < 0.003). The increased weight gain was associated with a slightly larger daily food intake, with total food consumption from weaning to the time the animals were killed being significantly greater (Ctl: 708 g; SmL: 20.9 g; P < 0.001). This increase in weight was correlated with a significant rise in circulating leptin levels (Ctl: 4.8 ± 0.5 ng/ml; SmL: 2.8 ± 0.4 ng/ml; P < 0.001) but no change in adiponectin (Ctl: 27.7 ± 6.2 ng/ml; SmL: 20.9 ± 3.5 ng/ml), glucose (Ctl: 89.0 ± 5.9 mg/dl; SmL: 90.0 ± 4.2 mg/dl), or insulin (Ctl: 1.2 ± 0.3 ng/ml; SmL: 1.5 ± 0.6 ng/ml) levels.

The increase in bw and leptin levels in rats from small litters was coincident with higher hypothalamic GFAP levels (Fig. 1A; P < 0.01), with GFAP levels directly correlating with bw (R = 0.881; P < 0.002) and leptin levels (R = 0.414; P < 0.03). Vimentin (Fig. 1B; P < 0.05), a structural protein expressed in tanyocytes and immature or activated astrocytes, and actin levels (Fig. 1C; P < 0.0001) were increased, with no change in PCNA levels (Ctl: 100 ± 5.9; SmL: 100.5 ± 16.7% control).

Because astrocytes are also involved in the control of synaptic inputs (16), we analyzed the hypothalamic levels of specific synaptic proteins as an index of overall synaptic density. In rats with increased weight gain, the presynaptic protein synaptotagmin was increased (Fig. 1D; P < 0.0001), with no change in synapsin I (Ctl: 100 ± 2.3; SmL: 91.8 ± 5.9% control), syntaxin (Ctl: 100 ± 9.7; SmL: 116.3 ± 7.9% control) or PSD95 (Ctl: 100 ± 1.2; SmL: 92.0 ± 5.0% control).

There was no change in GFAP (Ctl: 100 ± 3; SmL: 94 ± 7% control) or vimentin (Ctl: 100 ± 3; SmL: 86 ± 10% control) levels in the cerebellum, suggesting that astrocytes are not equally affected in all brain regions.

Chronic leptin treatment

Because leptin levels were increased in rats from litters of four pups and astrocytes express leptin receptors (17, 18, 20), we hypothesized that chronic exposure to increased leptin may be involved in the observed glial changes. Two weeks of leptin treatment [intracerebroventricular (icv)] significantly decreased the total weight gain (control: 16.7 ± 3.7 g; leptin: 2.2 ± 5.7 g; P < 0.05), although the difference in weight gain during the last part of the treatment was not significant (last 3 d of treatment: control, 5.5 ± 1.3 g; leptin, 4.8 ± 1.0 g). This suggests that at least some of the rats may be becoming resistant to the leptin treatment by the end of the study, which is supported by the fact that at the time the animals were killed, mean hypothalamic phosphorylated STAT3 (tyr705) levels were elevated compared with controls (control: 100 ± 7.8; leptin: 129.3 ± 10.1% control; P = 0.07), but this rise did not reach statistical significance.

There was no significant difference in serum leptin (control: 4.34 ± 1.06 ng/ml; leptin: 7.47 ± 4.57 ng/ml), glycemia (control: 92.9 ± 2.4 mg/dl; leptin: 93.0 ± 1.2 mg/dl), or insulin levels (control: 1.03 ± 0.29 ng/ml; leptin: 0.89 ± 0.45 ng/ml) at the time the animals were killed.

As observed in overweight rats, there was a significant increase in both GFAP (Fig. 2A; P < 0.05) and vimentin (Fig. 2B; P < 0.01) in the hypothalamus of leptin-treated rats. In contrast, actin levels were decreased (Fig. 2C; P < 0.001). In leptin-treated rats (Fig. 2, E and G), there were visibly more vimentin-labeled fibers and astrocytes in the...
arcuate nucleus (Fig. 2, D and F), with quantitative analysis showing a significant increase in the number of vimentin-positive fibers in response to leptin (Fig. 2H; \( P < 0.0001 \)).

Leptin modified the morphology of GFAP-positive astrocytes (Fig. 3, A and B). The mean astrocyte projection length was increased in leptin-treated rats (Fig. 3C; \( P < 0.001 \)), with no effect on the number of projections/astrocyte (Fig. 3D). Leptin decreased PCNA levels (control: 100 ± 5.5; leptin: 65.2 ± 3.8% control; \( P < 0.001 \)), but there was no change in the mean number of astrocytes in the arcuate nucleus (control: 11.7 ± 1.4; leptin: 11.8 ± 2.7 astrocytes/field). The effect of leptin on synaptic protein levels was inverse of that seen for GFAP and vimentin, with a decline in synapsin I (Fig. 4A; \( P < 0.01 \)), synaptotagmin (Fig. 4B; \( P < 0.02 \)), and PSD95 (Fig. 4D; \( P < 0.02 \)) levels and no change in the mean number of astrocytes in the arcuate nucleus (control: 11.7 ± 1.4; leptin: 11.8 ± 2.7 astrocytes/field).

Acute leptin treatment did not modulate serum leptin (C0: 0.91 ± 0.23 ng/ml; C1 h: 0.92 ± 0.13 ng/ml; L1 h: 3.68 ± 2.41 ng/ml; C6 h: 0.90 ± 0.31 ng/ml; L6 h: 2.60 ± 1.54 ng/ml) or insulin (C0: 0.59 ± 0.10 ng/ml; C1 h: 0.44 ± 0.01 ng/ml; L1 h: 0.79 ± 0.28 ng/ml; C6 h: 0.41 ± 0.06 ng/ml; L6 h: 0.45 ± 0.04 ng/ml) levels.

### Acute leptin treatment

Acute leptin treatment (icv) did not modulate serum leptin (C0: 0.91 ± 0.23 ng/ml; C1 h: 0.92 ± 0.13 ng/ml; L1 h: 3.68 ± 2.41 ng/ml; C6 h: 0.90 ± 0.31 ng/ml; L6 h: 2.60 ± 1.54 ng/ml) or insulin (C0: 0.59 ± 0.10 ng/ml; C1 h: 0.44 ± 0.01 ng/ml; L1 h: 0.79 ± 0.28 ng/ml; C6 h: 0.41 ± 0.06 ng/ml; L6 h: 0.45 ± 0.04 ng/ml) levels.

Changes in GFAP in response to an acute increase in central leptin levels were opposite those seen with chronic leptin. There was a significant effect of leptin (\( P < 0.0001 \)) and an interaction between treatment and time (\( P < 0.005 \)), with hypothalamic GFAP levels being reduced 1 h after leptin administration (Fig. 5A; \( P < 0.0002 \)) and increasing at 6 h in both the control and leptin groups. There was no effect on vimentin (C0: 100 ± 9.6; C1 h: 75.4 ± 21.2; L1 h: 79.9 ± 28.0; C6 h: 121.9 ± 13.8; L6 h: 78.6 ± 14.9% C0) or actin levels (C0: 100 ± 12.2; C1 h: 86.0 ± 12.8; L1 h: 124.8 ± 19.9; C6 h: 83.4 ± 9.8; L6 h: 74.7 ± 1.2% C0).
These rapid modifications in GFAP were associated with inverse changes in synapsin I (Fig. 5B) and syntaxin (Fig. 5C) levels with a significant rise in these presynaptic proteins at 1 h after leptin treatment, returning to control levels at 6 h. There was an effect of leptin treatment \((P < 0.005)\) and an interaction between treatment and time \((P < 0.05)\) on synapsin I levels with an effect of time \((P < 0.05)\) and treatment \((P < 0.02)\) on syntaxin levels. Synaptotagmin \((C0: 100 \pm 7.3; C1 h: 85.4 \pm 2.8; L1 h: 90.8 \pm 6.4; C6 h: 87.4 \pm 3.8; L6 h: 92.7 \pm 11.6\% C0)\) and PSD95 \((C0: 100 \pm 7.2; C1 h: 81.8 \pm 12.2; L1 h: 84.2 \pm 9.0; C6 h: 87.4 \pm 27.5; L6 h: 98.9 \pm 3.5\% C0)\) levels were unmodified.

There was no effect on cerebellar GFAP \((C0: 100 \pm 9.8; C1 h: 146.7 \pm 15.8; L1 h: 155.0 \pm 19.6; C6 h: 134.1 \pm 20.7; L6 h: 148.9 \pm 23.0\% C0), vimentin \((C0: 100 \pm 23.7; C1 h: 67.8 \pm 13.7; L1 h: 94.8 \pm 28.6; C6 h: 125.4 \pm 15.8; L6 h: 124.7 \pm 1.8\% C0), or actin \((C0: 100 \pm 17.5; C1 h: 112.0 \pm 5.6; L1 h: 110.8 \pm 19.4; C6 h: 108.3 \pm 9.8; L6 h: 117.7 \pm 7.2\% C0)\) levels were unmodified.

There was no effect on cerebellar GFAP \((C0: 100 \pm 9.8; C1 h: 146.7 \pm 15.8; L1 h: 155.0 \pm 19.6; C6 h: 134.1 \pm 20.7; L6 h: 148.9 \pm 23.0\% C0), vimentin \((C0: 100 \pm 23.7; C1 h: 67.8 \pm 13.7; L1 h: 94.8 \pm 28.6; C6 h: 125.4 \pm 15.8; L6 h: 124.7 \pm 1.8\% C0), or actin \((C0: 100 \pm 17.5; C1 h: 112.0 \pm 5.6; L1 h: 110.8 \pm 19.4; C6 h: 108.3 \pm 9.8; L6 h: 117.7 \pm 7.2\% C0)\) levels were unmodified.

**Discussion**

Astrocytes are known to participate in diverse neuroendocrine processes (13–15); however, their role in the control of appetite and energy balance has been largely ignored until recently. Likewise, although the presence of leptin receptors in these glial cells was reported more than a decade ago (20), their physiological relevance remains to be elucidated. An increase in the expression of leptin receptors in hypothalamic astrocytes was recently suggested...
to be involved in the onset of obesity (17, 18), with leptin’s inhibitory effect on feeding reported to be partially mediated through up-regulation of apolipoprotein E in these glial cells (27). Thus, determining the astroglial response to metabolic factors may be important for understanding the control of weight gain.

Early life events, including maternal and neonatal nutrition, affect the propensity to become obese in later life (1, 2, 19, 28). Reduction of litter size is a relatively natural procedure for inducing neonatal overfeeding and although maternal behavior is affected, the difference in food intake is the most important factor for the change in weight gain (29). Here we show that rats from smaller litters were significantly heavier than those from normal litters not only at weaning but also at 10 wk of age. This was due, at least in part, to a small daily increase in food intake with the cumulative intake being significantly higher in overweight rats. Although overweight, these rats were not hyperinsulinemic as might be expected, possibly because they were not fasted at the time they were killed. However, in a previous report using a similar protocol, mice from small litters had a significant increase in body weight, but fasting insulin levels were not affected unless the animals were subjected to a high-fat diet (30). Thus, it is also possible that development of insulin resistance is delayed in this animal model because these animals were young adults or that a change in diet and further weight gain are required (30). However, leptin levels were significantly increased as previously reported (30).

The increased weight gain in rats with neonatal overnutrition was associated with a rise in hypothalamic levels of the glial structural proteins GFAP and vimentin. Because these overweight rats were hyperleptinemic, with other circulating factors not being affected at this time, and astrocytes are reported to express the leptin receptor (17, 18, 20), it follows that this cytokine could be involved in the modulation of hypothalamic glial cells. Indeed, chronic central leptin infusion increased both GFAP and vimentin, similar to that observed in overweight rats. Furthermore, leptin induced structural changes in astrocytes...
of the arcuate nucleus, increasing the mean projection length, and reduced overall synaptic protein levels. In diverse neuroendocrine systems, changes in glial coverage of hypothalamic neuronal perikarya have been shown to be inversely related to modifications in the number of synaptic inputs (15). Although synaptic inputs were not counted here, proteins used as indirect indices of synaptic contact density (31) were altered, suggesting that this inverse relationship may exist in response to leptin. Furthermore, because levels of the postsynaptic protein PSD95 were decreased, at least part of the decline in synaptic contacts could correspond to glutamatergic synapses (32).

In contrast to chronic leptin treatment, acute leptin administration reduced hypothalamic GFAP and increased synaptic protein levels. This opposite but also inverse relationship between GFAP and synaptic proteins supports the argument that astrocytes may participate in leptin-induced synaptic remodeling. Acute leptin treatment did not affect all synaptic proteins, but one would not expect the entire synaptic structure to be dismantled if this process can be rapidly reversed once leptin, or other signals, return to baseline. Thus, postsynaptic structures, such as PSD95, would not be expected to change acutely but possibly only disconnected from the presynaptic input. In contrast, synapsin I, which regulates the number of synaptic vesicles available for release by binding synaptic vesicles to the cytoskeleton, thus modulating their migration to the presynaptic membrane (31), is more likely to be rapidly altered. Hence, leptin may rapidly increase the number of active synapses or the quantity of synaptic vesicles available for release, with the retraction of astrocytic processes possibly being involved. In contrast, chronic leptin exposure could induce long-term effects on synaptic structures that cannot be rapidly normalized. Indeed, chronic, but not acute, leptin treatment modified hypothalamic actin levels, and rearrangement of actin filaments in different brain regions has been associated with modifications in numbers of synaptic contacts and dendritic spines (33–35).

Hypothalamic GFAP levels changed throughout the day in control rats. These fluctuations, as well as glial coverage of neurons, have been previously described in the suprachiasmatic nucleus and are hypothesized to be involved in the control of circadian rhythms (36, 37). Whether these modifications in glial structural proteins are also involved in circadian changes in metabolic responses (38) remains to be determined.

Although GFAP levels increased, a decrease in hypothalamic synaptic protein levels was not observed in overweight rats. This could be due to the numerous modifications in circulating and central factors that occur with weight gain, with these possibly having opposing forces on synaptic densities. Indeed, leptin and ghrelin have opposite effects on synaptic inputs, both rapidly increasing some specific synapses and decreasing others (5). Thus, it is possible that subtle changes in synaptic densities take place on specific neuronal populations and these cannot be detected by the methods used here. Glial structural changes are not only involved in modifications of the number of synaptic inputs but can also affect synaptic efficacy due to their participation in glutamate clearance and by controlling extracellular ionic concentrations thus changing neuronal excitability (39). Astrocytes also release neuroactive substances, or gliotransmitters, which include glutamate, γ-aminobutyric acid, D-serine, and ATP, among others (39). Indeed, long-term potentiation in the hippocampus is reported to depend on calcium dependent D-serine release from astrocytes (40). Thus, the leptin-induced rise in calcium in hypothalamic astrocytes (17) could play a role in synaptic remodeling or efficiency as well as direct release of neuroactive substances.

Different authors have reported that astrocytes express the leptin receptor in vivo (17, 18, 20), but this matter remains a point of debate, and the leptin-induced glial structural changes observed here could in fact be mediated through actions on other cell types. In primary astrocyte cultures, leptin receptor mRNA was detected and increased in response to leptin, suggesting that this receptor may be present in astrocytes, at least under some conditions. However, cells removed from their normal physiological environment may or may not represent their in vivo phenotype. The fact that modifications in GFAP levels in response to leptin were similar in vivo and in vitro, with a decrease 1 h after an acute icv bolus of leptin and normalization at 6 h and then increasing at 24 h as observed in response to chronic icv leptin treatment, suggests that there may indeed be a direct effect on this cell type. However, further investigation is required to confirm this possibility and to determine the mechanisms involved.

Not all in vivo and in vitro effects of leptin were similar because no change in vimentin or actin levels was found in vitro. Thus, the in vivo changes in response to leptin or being overweight may be due to effects on other cell types, such as actin in neurons, or be an indirect effect of leptin on astrocytes. Because vimentin is a marker of both activated and immature astrocytes, the fact that it did not increase in vitro suggests that these cells were not activated and did not proliferate, which is supported by no change in PCNA levels, even in response to the elevated dose of leptin used in these studies.

Obesity is considered to be a state of chronic inflammation with alterations in the levels of various cytokines (41). Moreover, hypothalamic inflammation has recently been suggested to actively participate in the development
of obesity and central insulin and leptin resistance in response to a high-fat diet (12, 19, 42, 43). Free fatty acid-induced central inflammation is not thought to be a direct effect on neurons but is suggested to be mediated through other cell types such as astrocytes that can be activated in response to cytokines and participate in the inflammation response (12, 44, 45). Astrocytes were probably activated in both overweight and chronic leptin treated rats because vimentin levels were increased and cell proliferation markers were either unchanged or decreased. Because leptin is a cytokine and the amount of leptin infused in the studies reported here is expected to result in central levels well above those thought to occur under physiological circumstances, caution in the interpretation of these results should be used. However, hypothalamic vimentin levels were also increased in overweight rats, suggesting that astrocyte activation may indeed be a normal response to physiologically or pathophysiologically elevated levels of leptin or to other changes that occur with weight gain. Moreover, a large acute bolus of leptin modified levels of GFAP but not vimentin, indicating that although astrocyte morphology can be rapidly modified chronic exposure to increased leptin may be necessary to activate astrocytes.

In obese subjects leptin is chronically elevated, but they also exhibit leptin resistance (46–48), and the system that transports leptin from the circulation to the central nervous system is reported to be saturated (49). Whether activated astrocytes play a role in this process remains to be elucidated, but the increased expression of leptin receptors in hypothalamic astrocytes in obesity is suggested to participate in leptin transport, either from the circulation to the brain or within the brain itself (18). Thus, changes in astrocyte activation, morphology, and/or phenotype could modulate the sensitivity of the hypothalamus to leptin. The fact that glial proteins were unaffected in the cerebellum suggests that the modifications in hypothalamic astrocytes are associated with their neuroendocrine role. Indeed, it has become clear in recent years that astrocytes throughout the brain constitute specific subpopulations with different physiological functions (10).

In summary, hypothalamic astrocytes respond to changes in leptin levels, with acute and chronic exposure having differential effects. Acute leptin treatment induces rapid synaptic reorganization in the hypothalamus, increasing inhibitory and decreasing excitatory inputs to orexigenic neurons with the opposite effect on anorexigenic neurons (5), and the results reported here suggest that astrocytes could participate in this process. Furthermore, whether morphological changes in astrocytes are involved in the development of central leptin resistance and whether these structural modifications are rapidly reversible with weight loss or decreased central leptin levels remain to be determined. Thus, it is clear that understanding the glial response to metabolic factors is important for our comprehension of the hypothalamic response to increased weight gain.

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