Commentary

MOLECULAR METABOLISM

AstroGenesis: And there was leptin on the sixth day

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In the central nervous system (CNS), the hypothalamus plays a fundamental role in controlling food intake and energy expenditure as well as glucose homeostasis. Alterations in this control can lead to obesity, glucose intolerance and type 2 diabetes in both rodents and humans [1]. The past two decades have witnessed major advances in our understanding of the control of energy balance by hypothalamic neuronal populations. These neurons respond to peripherally-derived metabolic signals to elicit adaptive behavioral and physiological responses in order to maintain body weight. Leptin, the most notorious adipose-derived hormone, targets neurons of the hypothalamus, notably orexigenic agouti-related protein (AgRP) and anorexigenic pro-opiomelanocortin (POMC) neurons of the arcuate nucleus (ARC), to decrease feeding and increase energy expenditure. Leptin binding to its receptor (LepR) in ARC neurons regulates neuronal activity and synaptic plasticity by activating signaling pathways that include phosphonositide 3-kinase (PI3K), extracellular signal-regulated kinases (ERK), AMP-activated protein kinase and signal transducer and activator of transcription 3 (STAT3) [1]. Leptin deficiency or loss-of-function mutations in the LepR lead to hyperphagia and obesity in both rodents and humans illustrating the critical role of leptin signaling in central control of energy homeostasis. In addition to the well-established actions of leptin on metabolic neurocircuits, non-neuronal cells of the hypothalamus have recently emerged as new targets and mediators of leptin effects on energy balance [2,3]. In this issue of Molecular Metabolism, Rottkamp et al. add a brand new element to the leptin signaling puzzle with demonstration that leptin enhances astrocytes proliferation in the hypothalamus during postnatal development [4].

During CNS development, neurons and glial cells are sequentially generated. Neurogenesis occurs during prenatal brain development and most neurons of the adult brain are already produced at birth. In contrast, glial cells are generated after the end of the first postnatal week and account for more than half of brain cells in adults. Astrocytes, the most abundant glial cells in the brain, provide anatomical and metabolic support for neurons. In addition, they regulate several neuronal processes including proliferation, differentiation and synaptogenesis. Recent evidence suggests that hypothalamic astrocytes play an important role in metabolic sensing, energy balance regulation and obesity (reviewed in [5]). Interestingly, astrocytes of the hypothalamus express the LepR, and leptin modulates astrocytes morphology as well as glutamate and glucose transport [6], suggesting that leptin signaling in hypothalamic astrocytes may regulate neuronal plasticity and excitability. The physiological role of leptin signaling in astrocytes was recently established by Kim et al. [2] who demonstrated that LepR ablation (exon 17) in adult glial fibrillary acidic protein (GFAP)-astrocytes using a tamoxifen inducible GFAP-CreERT2 mouse blunts the anorectic action of leptin. This phenotype was associated with reduced glial coverage and altered synaptic inputs to AgRP and POMC neurons in the ARC. Consistent with the diminished anorectic effects of leptin in mice lacking LepR in GFAP-adult astrocytes, Wang et al. [3] reported that LepR ablation (exon 1) in GFAP-astrocytes early during development (embryonic knockout using non-inducible GFAP-Cre mice) reduces leptin-induced STAT3 phosphorylation in hypothalamic GFAP-astrocytes and increases the susceptibility to diet-induced obesity. However, it is important to mention that in ventricular zones, GFAP is also a marker of radial glia, which gives rise to astrocytes, neurons and oligodendrocytes during CNS development. Thus, it is possible that LepR was ablated in ARC neuronal subpopulations and other cell types leading to confounding effects on the phenotype of the animals during high-fat feeding. Nonetheless, these studies highlight a key role for astroglial leptin signaling in the organization of ARC neurocircuits, leptin suppression of feeding and etiology of obesity.

In addition to its effect on adult neurons and astrocytes in the hypothalamus, leptin acts as neurotrophic factor during postnatal development promoting neurites outgrowth of POMC and AgRP neurons [7]. However, the role of leptin signaling in astrocytes plasticity and astrogenesis during neonatal development remains unclear. This question is important given that astrocyte proliferation in young pups is concomitant to the postnatal surge in leptin levels which begins approximately on the sixth day after birth and declines after weaning. The study by Rottkamp et al. [4] is the first to suggest a causal link between the postnatal leptin surge and astrocyte proliferation in the developing hypothalamus. Using exogenous leptin treatment and inducible LepR loss-of-function, Rottkamp et al. demonstrate that

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elevated leptin levels in the postnatal period potentiate astrogenesis in the hypothalamus in a LepR-dependent manner in GFAP-astrocytes.

First, they show that GFAP immunostaining is increased in the ARC of obese mice models characterized by high leptin levels but not in leptin deficient mice (*ob*/*ob*). Then, using the mitotic marker BrdU and GFAP immunostaining, they demonstrate that daily leptin treatment of pups from P8 to P12 increases the number of parenchymal BrdU/GFAP double positive cells in adult mice. To exclude the potential confounding effect of exogenous leptin on GFAP expression, similar experiments were performed in mice carrying both a tamoxifen-inducible GFAP-CreERT2 and Cre-activable-tdTomato reporter. In this model, leptin treatment increased the number of dtTomato-positive cells and BrdU/dt-Tomato double positive cells in the ARC thereby supporting the idea that leptin enhances astrocyte proliferation.

Finally, they elegantly established a causal link between astroglial leptin signaling and astrocyte proliferation in the ARC. To this end, tamoxifen-inducible GFAP-CreERT2 mice carrying the dt-Tomato reporter were crossed with LepR *floxed* mice (exon 17). Disruption of LepR in GFAP-astrocytes at P4-P5 decreased the number of parenchymal BrdU/dt-Tomato double positive cells in the ARC in the absence of exogenous leptin. Although recombination efficiency in adult mice carrying the GFAP-CreERT2 transgene has been previously validated [2], the level of LepR deficiency in GFAP-astrocytes was not analyzed in pups after tamoxifen injections. While this could be seen as a limitation, the expression of dt-Tomato combined to a decreased number of proliferating cells support the idea that recombination occurred in GFAP-astrocytes.

Together, their findings strongly suggest that leptin stimulates astrogenesis via LepR in GFAP-astrocytes in the developing ARC. This work adds important insights on leptin action in the hypothalamus and suggests that the hormone directly contributes to the establishment of a functional network of astroglial cells in the hypothalamus.

These findings raise important questions related to the role of astrocytes in hypothalamic neurocircuits organization and control of energy homeostasis. Astrocyte populations are highly heterogeneous throughout the CNS, even within the same region, as exemplified by differences in morphology and expression of astroglial markers (GFAP, S100β, GLT-1, GLAST and Aldh1l1) [8]. As such, it would be interesting to determine if, in addition to its effect on proliferation, leptin favors the differentiation of a particular astrocyte subtype in the hypothalamus that may in turn regulate specific neuronal processes.

Another important aspect will be to determine whether leptin-responsive astrocytes contribute to the formation of the neuronal circuitry during postnatal development of the hypothalamus. The authors show that the number of POMC and AgRP neurons in three weeks old mice was not affected by LepR-deficiency in GFAP-astrocytes. However, this does not rule out the possibility that synaptic inputs and axonal projections of AgRP and POMC neurons to other hypothalamic nuclei may be affected in young animals leading to metabolic perturbations later in life. This idea is supported by the findings of Kim et al. showing that LepR ablation in adult astrocytes alters the organization of synaptic inputs on AgRP and POMC neurons [2]. In the same line, it would be interesting to investigate if leptin regulates astrogenesis and formation of neurocircuits during development in other hypothalamic nuclei (such as the VMH) and/or brain regions (such as mesolimbic reward system) that are known to play a key role in central leptin action on energy balance.

Which signaling pathway downstream of LepR potentiates astrogenesis in the hypothalamus?

The results of Rottkamp et al. suggest that leptin-induced STAT3 phosphorylation is not altered in GFAP-cells in astroglial LepR-ablated mice. This suggests that leptin potentiation of astrogenesis is STAT3-independent. However, it is important to note that the GFAP-cells analyzed were periventricular cells lining the 3rd ventricle and not parenchymal cells in the ARC. Thus, it is possible that the GFAP-periventricular cells are not proliferating at this developmental stage or belong to a subtype of GFAP-positive glial cells, possibly radial glia, in which proliferation depends on an alternate leptin signaling pathways such as PI3K or ERK.

As such, further studies will be required to determine whether or not leptin potentiation of astrogenesis is STAT3-dependent in ARC parenchymal astrocytes and assess the implication of other potential signaling pathways.

Additionally, it is conceivable that leptin is not the only metabolic signal regulating postnatal astrogenesis and its action is part of a concerted hormonal response. For instance, the orexigenic hormone ghrelin antagonizes the neurotrophic influence of leptin and maturation of hypothalamic neuronal feeding circuits during neonatal development [9]. Whether a similar interaction occurs in hypothalamic astrocytes to regulate proliferation or plasticity has yet to be investigated.

Finally, the control of astrogenesis by leptin reported by Rottkamp et al. [4] may have important implications in the context of maternal obesity, which is known to predispose offspring to metabolic perturbations and obesity in adulthood. Offspring of obese rats exhibit an amplified and prolonged postnatal leptin surge which is associated with central leptin resistance and hyperphagia later in life [10]. In addition, neonatal overnutrition increases the number of GFAP-positive cells in the hypothalamus and induces overweight in adult rats [6]. Together, these findings make it tempting to speculate that increased proliferation of astrocytes in response to an amplified leptin surge in neonates from obese dams may underlie, at least in part, the developmental programming of obesity.

The study by Rottkamp et al. suggesting that leptin acts as a gliotrophic signal in the developing hypothalamus adds a novel and important piece to the growing literature on the role of non-neuronal hypothalamic cells in energy balance. It paves the way for future studies aimed at defining the cellular features and contribution of astrocytes in the organization and activity of metabolic neurocircuits in the hypothalamus. Such studies will be critical to better understand the complex interplay between astrocytes and neurons and its role in the etiology of metabolic diseases.

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CONFLICT OF INTEREST

None declared.

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