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BRAIN RESEARCH

Research Report

Leptin-dependent STAT3 phosphorylation in postnatal mouse hypothalamus

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ABSTRACT

Leptin, a hormone produced by adipose tissue, reduces food intake and boosts energy expenditure via activation of the JAK2-STAT3 signalling pathway in adult mammal hypothalamic neurons. It is found in blood early after birth, peaking around postnatal day (P) 10. The hypothalamus of neonatal mice administered intraperitoneal leptin (3 mg/kg of body weight) was investigated for phospho-STAT3-positive cells to gain insights into the timing of maturation of the leptin signal transduction system. Leptin responsiveness was first detected in arcuate nucleus, where it was faint at P1 and evident from P5. It was then identified in medial preoptic area, anterior hypothalamus, retrochiasmatic area, dorsomedial nucleus and premammillary nucleus from P7, and in ventromedial nucleus and lateral hypothalamus from P11. From P13 onwards, hypothalamic P-STAT3 staining was indistinguishable from that of adult mice. Significant hypothalamic STAT3 activation was also detected by Western blotting at P11 and P15. The level of activation seen in adults was comparable to that observed at P15 although, remarkably, leptin-induced feeding reduction is observed only after the fourth postnatal week. Neuronal and glial markers and double-labelling immunohistochemistry showed that leptin-stimulated hypothalamic cells that had already reached their final position in a given area or nucleus were neurons; however, leptin responsiveness preceded positivity for the neuronal markers, suggesting a not fully differentiated status. Interestingly, leptin also increased P-STAT3 and c-Fos immunoreactivity in a distinctive and transient (from P5 to P13) cell population found in the dorsal part of the third ventricle and in subependymal position. These cells did not express mature or immature neuronal or glial markers. Their ultrastructural appearance, though suggestive of differentiating cells, was not conclusive for a specific phenotype.

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Abbreviations: AHA, anterior hypothalamic area; ARC, arcuate nucleus; BBB, blood–brain barrier; DMH, dorsomedial hypothalamic nucleus; i.p., intraperitoneal; JAK, Janus kinase; LHA, lateral hypothalamic area; MPO, medial preoptic area; P, postnatal day; STAT3, signal transducer and activator of transcription 3; PMN, premammillary nuclei; PVH, paraventricular nucleus; P-STAT3-IR, phospho-STAT3 immunoreactivity; RCh, retrochiasmatic area; VMH, ventromedial hypothalamic nucleus

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1. Introduction

Leptin, the product of the *ob* (obese) gene, is a circulating peptide produced primarily by adipocytes in proportion to the body's fat energy stores (Zhang et al., 1994). In adult animals leptin is an important regulator of the energy balance, mainly through its actions in the brain in relation to food intake and energy expenditure (Friedman, 2002; Coll et al., 2007).

Leptin receptors (Ob-R) are the product of several alternatively spliced forms of the *db* (diabetes) gene (Chen et al., 1996; Lee et al., 1996). They are found in several tissues and organs, including a number of brain areas (Tartaglia et al., 1995; Fei et al., 1997). One splice variant, Ob-Rb, encoding a transmembrane protein with a longer cytoplasmic domain, is often referred to as the "long" or "functional form" of the receptor. Ob-Rb is highly expressed in the hypothalamus (Lee et al., 1996; Mercer et al., 1996; Schwartz et al., 1996; Fei et al., 1997; Elmquist et al., 1998). Mutations in the protein result in an obese phenotype (*db/db* mouse and Zucker *fa/fa* rat), demonstrating that leptin signalling in the hypothalamus via this receptor is required for normal energy homeostasis in adult rodents (Chen et al., 1996; Lee et al., 1996). Ob-Rb belongs to the family of cytokine receptors that lack the intrinsic catalytic kinase domain and function through cytoplasmic kinases. The cytoplasmic chain of Ob-Rb has a consensus amino acid sequence that is involved in the activation of Janus kinase-signal transducer and activator of transcription (JAK-STAT) tyrosine kinases and specifically activates STAT3 proteins (Baumann et al., 1996; Vaisse et al., 1996). Phosphorylated STAT3 proteins dimerize and translocate to the cell nucleus of leptin-sensitive neurons, where they bind to DNA, activate gene transcription and regulate neuroendocrine, autonomic and behavioural responses (Myers, 2004). Given the absence of specific Ob-Rb antibodies, detection of nuclear phospho-STAT3 immunoreactivity (P-STAT3-IR) after a single leptin injection has been proposed as a reliable neuroanatomical tool for the functional mapping of central leptin actions and the characterization of leptin-responsive, Ob-Rb-bearing neurons (Hubschle et al., 2001).

While it is generally accepted that leptin has an adipostatic action in adult animals, inhibiting food intake and boosting energy expenditure through the central activation of the JAK2-STAT3 signalling pathway (Sweeney, 2002; Myers, 2004), its physiological role in the rodent postnatal period, i.e. between birth and weaning, is unclear. Circulating leptin is detectable in pups as early as the first few days after birth, its level progressively rising to a peak around postnatal day 10 (P10), and then slowly declining to the concentration found in adults (Devaskar et al., 1997; Ahima et al., 1998; Yura et al., 2005). Although the neonatal mouse brain expresses all receptor isoforms (Proulx et al., 2002), administration of intraperitoneal (Mistry et al., 1999; Proulx et al., 2002) or intracerebroventricular (Mistry et al., 1999) leptin fails to reduce food intake and body weight during lactation and until after weaning. In addition, leptin-deficient rodent models (*ob/ob* mouse, *db/db* mouse and *fa/fa* rat) do not show hyperphagia or increased fat accumulation and body weight before the fourth week of postnatal life (Boissonneault et al., 1978; McLaughlin and Baile, 1981; Mistry et al., 1999). Leptin does not therefore appear to affect food intake and body weight in suckling mice, whereas it may have a neurotrophic role in postnatal hypothalamus (Bouret et al., 2004a), cortical neurons (Valerio et al., 2006) and hippocampus (Walker et al., 2007).

The present study was designed to determine whether leptin modulates STAT3 signalling in the postnatal hypothalamus. To do this, mice in the first three weeks of life received intraperitoneal (i.p.) mouse recombinant leptin (3 mg/kg of body weight); the pattern of leptin-induced P-STAT3 activation was evaluated by immunohistochemistry and the degree of STAT3 activation was quantified by Western blotting

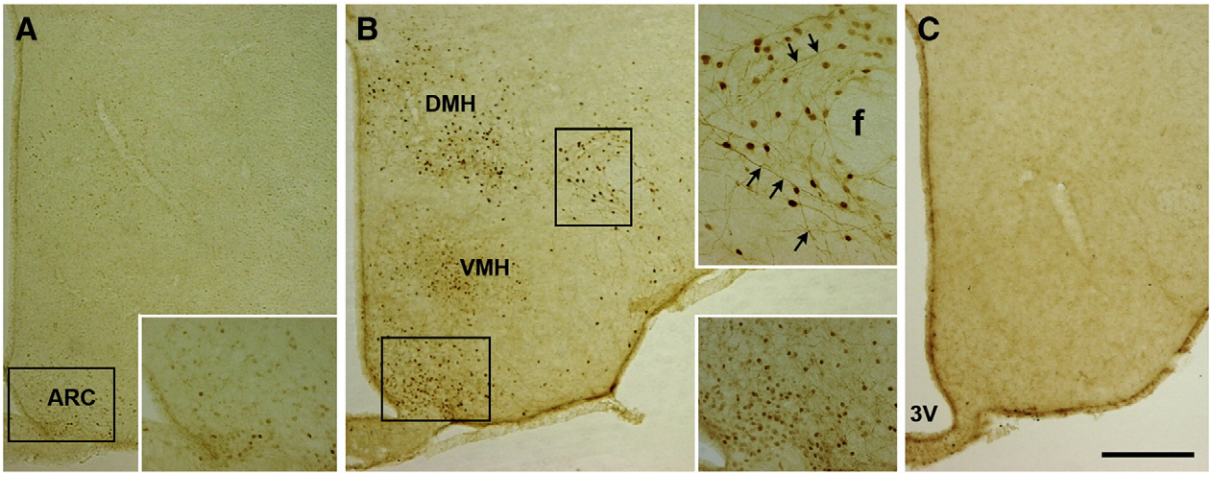

Fig. 1 – P-STAT3 immunohistochemical expression in adult mouse hypothalamus. Unstimulated mouse (A): P-STAT3 is detectable only in the nuclei of neurons located in the arcuate nucleus (ARC). Leptin-treated mouse (B): P-STAT3 expression increases in the ARC, the dorsomedial (DMH) and ventromedial (VMH) hypothalamic nuclei and the perifornical area, where P-STAT3-positive neural projections also become visible (top inset, arrows). *db/db* mouse (C): leptin treatment fails to activate the JAK-STAT3 signalling pathway. Insets are enlargements of the corresponding framed areas. Abbreviations: f, fornix; 3V, third ventricle. Bar: A–C = 200 μm; insets = 120 μm.