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Hypothalamic inflammation and thermogenesis: the brown adipose tissue connection

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Abstract Hypothalamic inflammation and dysfunction are common features of experimental obesity. An imbalance between caloric intake and energy expenditure is generated as a consequence of this inflammation, leading to the progressive increase of body adiposity. Thermogenesis, is one of the main functions affected by obesity-linked hypothalamic dysfunction and the complete characterization of the mechanisms involved in this process may offer new therapeutic perspectives for obesity. The brown adipose tissue is an important target for hypothalamic action in thermogenesis. This tissue has been thoroughly studied in rodents and hibernating mammals; however, until recently, its advocated role in human thermogenesis was neglected due to the lack of substantial evidence of its presence in adult humans. The recent demonstration of the presence of functional brown adipose tissue in adult humans has renovated the interest in this tissue. Here, we review some of the work that shows how inflammation and dysfunction of the hypothalamus can control brown adipose tissue activity and how this can impact on whole body thermogenesis and energy expenditure.

Keywords Obesity · Hypothalamus · Mitochondria · Uncoupling protein · Autonomic nervous system · TNFα · BAT

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Introduction

To date, obesity affects more than 300 million human beings in the world and this number is expected to increase to more than one billion by the year 2030 (Kelly et al. 2008). Besides its direct impact on life quality, obesity is a major risk factor for highly lethal diseases such as type 2 diabetes mellitus, hypertension, atherosclerosis and certain types of cancer (Anandacoomarasamy et al. 2008; Daniels 2009; Haslam and James 2005; Tsiros et al. 2009).

Behavioral approaches attempting to correct nutritional overload and increase physical activity have generally failed to revert or even slow-down the rate of progression of this disease. In addition, the few pharmacological compounds currently approved for use have a number of side effects and lead to body mass reduction that reaches 15% at most (Hainer et al. 2008; Neovius and Narbro 2008; Ross and Bradshaw 2009). At present, massively obese patients can be treated by bariatric surgery achieving fairly good body mass and metabolic control; however, this is an invasive and expensive method that should be employed only for patients with severe co-morbidities (Ross and Bradshaw 2009; Tice et al. 2008). Thus, it is believed that only the complete elucidation of the mechanisms leading to obesity may provide the means for the development of more effective and safer therapeutic approaches.

Recent studies have placed the hypothalamus in a central position in the pathophysiology of obesity. Although studies dealing with the roles played by the hypothalamus in the control of energy homeostasis began more than 70 years ago, it was only after the identification of leptin and of its cognate receptor, LepR, in 1994/1995 that great advance was obtained in this field. Leptin, the product of the *ob* gene, is produced mostly in the adipose tissue, in direct proportion to whole body adiposity (Zhang et al.

1994). It acts in the hypothalamus to activate LepR, leading to the regulation of neurotransmitter expression and release, which results in the control of feeding and thermogenesis (Chua et al. 1996; Tartaglia et al. 1995). The actions of leptin can be quantitatively determined in experimental animals by at least two distinct methods. At the functional level, leptin can exert a potent suppression of food intake when injected in the hypothalamus. In most strains of rodents, the reduction of spontaneous food intake in lean animals occurs during a time window, spanning from two to 12 h, and results in an up to 50% reduction in caloric intake (Myers et al. 2010). At the molecular level, leptin action can be determined by the evaluation of STAT3 tyrosine phosphorylation by immunoblot (Carvalheira et al. 2001). Additionally, leptin can also modulate thermogenesis, which can be demonstrated as changes in body temperature and respirometric parameters. Although a number of studies have explored these effects of leptin, it is not as routinely employed as the determination of the inhibition of feeding and the molecular activation of STAT3, as described above.

Defining leptin and insulin as adiposity and thermogenesis stimulating factors

Whilst leptin was at first speculated to be a potential therapeutic tool for obesity, it was soon realized that most obese subjects were hyperleptinemic and resistant to the actions of leptin. In experimental obesity, hypothalamic resistance to leptin is one of the first events to take place (Prada et al. 2005). This has an impact on both caloric intake and energy expenditure, and is currently regarded as the main molecular determinant of increased body adiposity (Araujo et al. 2010).

To date, only leptin and, to a minor extent, insulin, are considered primary adiposity factors. In fact, some studies have shown that the activity of leptin in the hypothalamus is enhanced by insulin (Carvalheira et al. 2001; Niswender et al. 2003, 2004). Both the physiological response to leptin, as determined by the suppression of feeding, as well as the transduction of its signal, as determined by activation of PI3K or STAT3, are significantly increased if leptin and insulin act together. Thus, the existence of a converging signaling pathway has been proposed, where this pathway is thought to be involved in the regulation of neuronal firing and neurotransmitter expression, which play a central role in the physiological regulation of feeding and thermogenesis and in the pathological processes that lead to obesity (Niswender and Schwartz 2003).

Only a few studies have evaluated the direct role of leptin and insulin in the control of thermogenesis. These actions can be detected at the functional level, as the increased O_2 consumption/CO_2 production in respirometric assays, as well as at the neurophysiologial level, as the control of neurotransmitter expression in the hypothalamus. Although there are still some controversies regarding the direct impact of leptin and insulin on O_2 consumption/CO_2 production, most studies have detected no changes, or only minor modifications of these parameters in rodents (Romanatto et al. 2007, 2009; Vaanholt et al. 2008). However, a number of groups have reported the increased expression of uncoupling protein 1 (UCP1), a key protein involved in thermogenesis, in the brown adipose tissue (BAT) of rodents treated icv with either leptin or insulin, strongly implying the hypothalamic actions of these hormones on thermogenesis (Minokoshi et al. 1999; Shek and Scarpace 2000; Wang et al. 1997). Moreover, the impact of leptin and insulin on thermogenic neurotransmitter expression reinforce their role in this physiological event (Elias et al. 1998; Wang et al. 1997). The reason why increased BAT activity following leptin or insulin hypothalamic action is not matched by increased respirometric parameters is unknown, but it may be due to the array of complex systemic effects of these hormones. In addition, it is also possible that the net effect of leptin and insulin on O_2 consumption/CO_2 production is small and, thus, not detectable by current methods. Therefore, further studies, and perhaps the refinement of the methods, will be necessary to solve this issue.

Hypothalamic inflammation and dysfunction in obesity

There are five molecular mechanisms currently known to promote leptin and/or insulin resistance in the hypothalamus: SOCS3, PTP1B, JNK, IKK and PKC-theta (Fig. 1). SOCS3 expression can be induced by both physiological and pathological conditions. SOCS3 belongs to the suppressor of cytokine signaling family of proteins and its expression can be induced by either leptin or insulin through the activation of STAT3 transcriptional activity (Howard and Flier 2006; Mori et al. 2004). In genetic and diet-induced obesity, increased levels of SOCS3 in hypothalamic neurons impair the leptin-mediated control of neurotransmitter expression, contributing to the development of the obese phenotype (Mori et al. 2004). The transient overexpression of SOCS3 impairs leptin signal transduction, while the hypothalamic knockdown of this protein protects against diet-induced obesity (Howard et al. 2004; Howard and Flier 2006).

PTP1B is overexpressed in the hypothalamus of obese rodents and is regarded as an important negative modulator of the hypothalamic adipostatic signals (Zabolotny et al. 2002). This protein impairs leptin and insulin signal transduction by dephosphorylating JAK2 and, thus, disrupting the mainframe of the adipostatic signal transduction

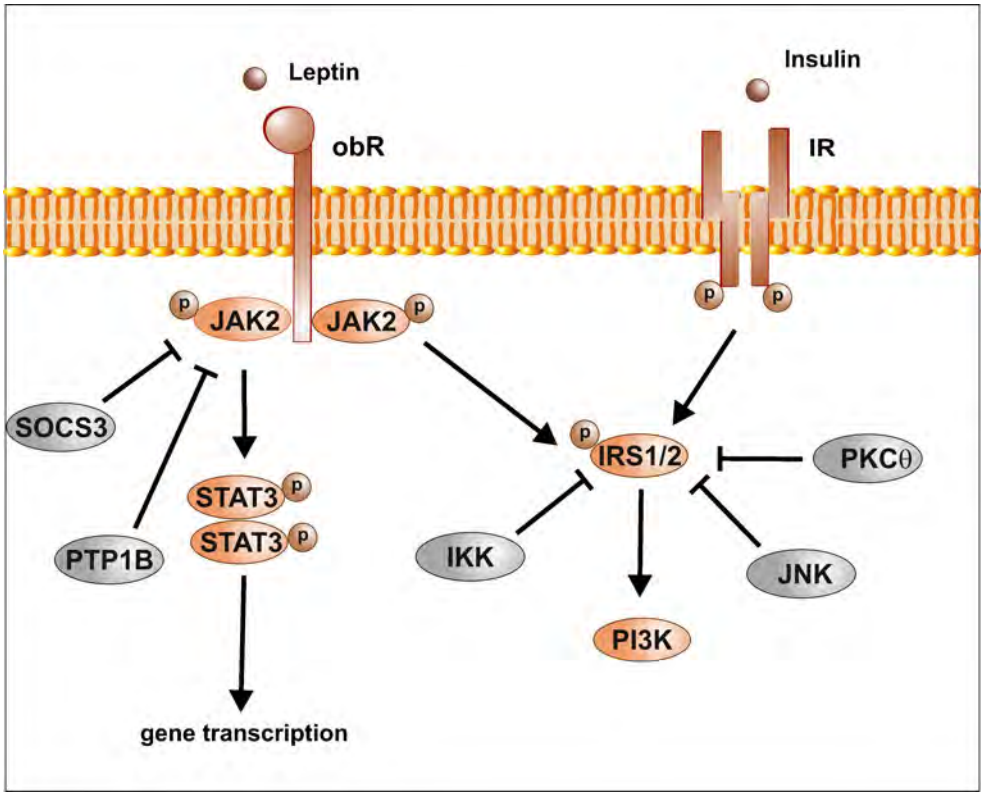

Fig. 1 Schematic representation of the currently known mechanisms leading to leptin and insulin resistance in the hypothalamus: IKK, JNK and PKC-theta can impair signal transduction by catalyzing inhibitory serine phosphorylation of important substrates (represented here by IRS proteins); PTP1B catalyzes the dephosphorylation of tyrosine residues, while SOCS3 physically blocks JAK2/STAT3 activation

system (Cheng et al. 2002; Zabolotny et al. 2002). The hypothalamic knockout of the PTP1B gene or its inhibition by an antisense oligonuleotide approach protects against diet-induced obesity (Bence et al. 2006; Picardi et al. 2008; Zabolotny et al. 2002). Moreover, the POMC neuron-specific deletion of the PTP1B leads to reduced adiposity and increased energy expenditure through a leptin-dependent mechanism (Banno et al. 2010).

c-Jun-N-terminal kinase (JNK) is a serine/threonine kinase, activated in response to a number of inflammatory and environmental factors (Davis 2000). In diet-induced obesity, there is an increased activation of JNK in the hypothalamus and the pharmacological inhibition of its activity corrects the defective leptin and insulin hypothalamic signal transduction, while attenuating the obese phenotype (De Souza et al. 2005). In addition, in JNK knockout mice, insulin signal transduction in the hypothalamus is enhanced (Unger et al. 2010).

The serine kinase IKK is one of the most important intracellular pro-inflammatory signal transducers (Israel 2010). It activates the transcription factor, NFκB, in response to a number of microbial, chemical and immunological stimuli leading to an increased inflammatory transcriptional activity (Israel 2010). Recent data have shown that, in the hypothalamus of obese rodents, IKK is atypically activated, and the hypothalamic targeting of its expression protects against diet-induced obesity. Moreover, the induced expression and activation of IKK in the hypothalamus leads to a leptin/insulin resistance state (Zhang et al. 2008).

Finally, a fifth mechanism contributing to the impairment of adipostatic signaling in the hypothalamus is the increased expression of the serine/threonine kinase PKC-theta (Benoit et al. 2009). Accordingly, the long-chain saturated fatty acid palmitate induces the increased expression of this protein, mostly in AgRP neurons of the arcuate nucleus. This leads to impaired hypothalamic insulin action and predisposes to increased adiposity. Site-specific knockdown of PKC-theta protects against diet-induced obesity (Benoit et al. 2009).

All the five major mechanisms leading to hypothalamic resistance to leptin and insulin can be activated by inflammatory factors. This is quite similar to the well-known events that lead to peripheral insulin resistance in type 2 diabetes (Hotamisligil 2010), suggesting that hypothalamic resistance to the adipostatic hormones may be only an extension of the whole body insulin resistance phenotype. In this context, obesity would begin by a discrete increase in the adipose tissue mass. Macrophages would then be recruited and trigger a local inflammatory response. Soluble inflammatory factors produced in the adipose tissue would then act systemically to activate inflammatory signaling pathways in insulin-sensitive tissues, resulting in whole-body insulin resistance. When reaching the CNS, the adipose tissue-produced inflammatory factors would activate local inflammation, inducing

leptin/insulin resistance and, thus, fostering obesity. However, most recent studies refute this view, using a number of different experimental approaches, that are introduced below.

During the induction of obesity by a western diet, the hypothalamus is the first tissue to become insulin-resistant, preceding by many weeks the installation of insulin resistance in the adipose tissue (Prada et al. 2005). The local inhibition of each of the five major mechanisms leading to leptin/insulin resistance in the hypothalamus is sufficient to reduce diet-induced obesity (Benoit et al. 2009; De Souza et al. 2005; Milanski et al. 2009; Mori et al. 2004; Picardi et al. 2008; Romanatto et al. 2009; Zhang et al. 2008). Moreover, the hypothalamic overexpression of some of the inducers of leptin/insulin resistance is sufficient to increase food intake and/or adiposity (Howard and Flier 2006; Unger et al. 2010; Zhang et al. 2008). In addition, while the systemic immunoneutralization of TNF-α by infliximab has no effect on body mass (Araujo et al. 2007), the hypothalamic use of this anti-TNF-α monoclonal antibody reduces body mass gain and increases thermogenesis (Romanatto et al. 2009). Finally, low-grade hypothalamic inflammation induced, by a very low dose of TNF-α can induce local resistance to leptin/insulin and modulate neurotransmitter expression towards an obesity-like pattern (Romanatto et al. 2007).

Thus, it seems reasonable to believe that hypothalamic inflammation is an important mechanism, leading to local leptin/insulin resistance, and that this phenomenon plays a major role in experimental obesity. In most instances, hypothalamic inflammation in obesity develops independently from systemic inflammation and, in fact, it may act as a determining factor for the installation of increased adiposity and systemic inflammation.

The impact of hypothalamic inflammation on thermogenesis

The current interest in the hypothalamic functional role in thermogenesis is mostly concentrated in the obesity field. However, during recent decades important data have been obtained from the research on sepsis and fever. It is now clear that, in obesity, sepsis and other acute inflammatory diseases, hypothalamus function is modulated by inflammatory factors. However, the outcomes of these inflammatory processes are divergent. During sepsis, inflammatory factors such as TNF-α, IL-1β, HMGB1 and LPS, among others, can act in the hypothalamus to induce fever, anorexia and body mass loss (Arruda et al. 2010; Gourine et al. 1998; Yang et al. 2005); whereas in obesity, TNF-α, and possibly other cytokines and inflammatory factors, act in the hypothalamus to reduce energy expenditure and increase caloric intake, leading to body mass gain (Romanatto et al. 2007, 2009).

Thus, one may question how similar factors and mechanisms, acting in the same anatomical region, produce such different outcomes? To provide some answers to this puzzle, we evaluated the effects of different amounts of TNF-α in a number of hypothalamic parameters related to the control of thermogenesis. An important concept to bear in mind when considering the impact of inflammatory factors on thermogenesis, is that systemic levels of inflammatory factors, such as TNF-α are up to 10-fold higher in sepsis than in obesity (Dandona et al. 1998; Kayacan et al. 2006). In the hypothalamus, differences in the local levels of this cytokine are barely 3-fold, when comparing tumor-bearing, obese and lean rodents (Arruda et al. 2011). However, even these small differences may affect neurotransmitter expression, feeding, responsiveness to leptin and insulin, body temperature, adiposity, and thermogenesis. With regard to the hypothalamic expression of neurotransmitters, while high levels of TNF-α produce an increment of the thermogenic neurotransmitters TRH and CRH, accompanied by the reduction of the anti-thermogenic NPY and MCH (Arruda et al. 2010), low levels of the cytokine reduce TRH and CRH (Arruda et al. 2011). These effects are accompanied by reduced feeding and adiposity in high TNF-α-treated animals (Arruda et al. 2010), and increased adiposity without a significant change in caloric intake in low TNF-α treated animals (Arruda et al. 2011). Moreover, while high levels of TNF-α in the hypothalamus increase body temperature and O_2 consumption/CO_2 production (Arruda et al. 2010), low levels have the opposite effect (Arruda et al. 2011).

The BAT is functionally modulated by hypothalamic inflammation

Both extremes of hypothalamic inflammation, sepsis and obesity, have an effect on BAT functional activity (Fig. 2). The first, and perhaps most remarkable evidence of this is the impact of high-concentration hypothalamic TNF-α on BAT morphology. Both macro- and microscopic changes are induced by this approach, which is mostly due to the reduced size of lipid droplets and increased number of mitochondria (Arruda et al. 2010). These changes are completely abrogated by BAT sympathetic denervation, suggesting that BAT activation by high hypothalamic TNF-α depends on the adrenergic system. Importantly, the denervation of BAT inhibited TNF-α-induced hyperthermia, suggesting that most of the thermogenetic activity seen in this condition was indeed due to BAT activation (Arruda et al. 2010).

Conversely, the icv injection of a low-dose of TNF-α produces significant reductions in the expressions of UCP1, cytochrome C, PGC-1α and Dio2 in BAT (Arruda et al. 2011). In addition, the icv injection of stearic acid (C18:0),

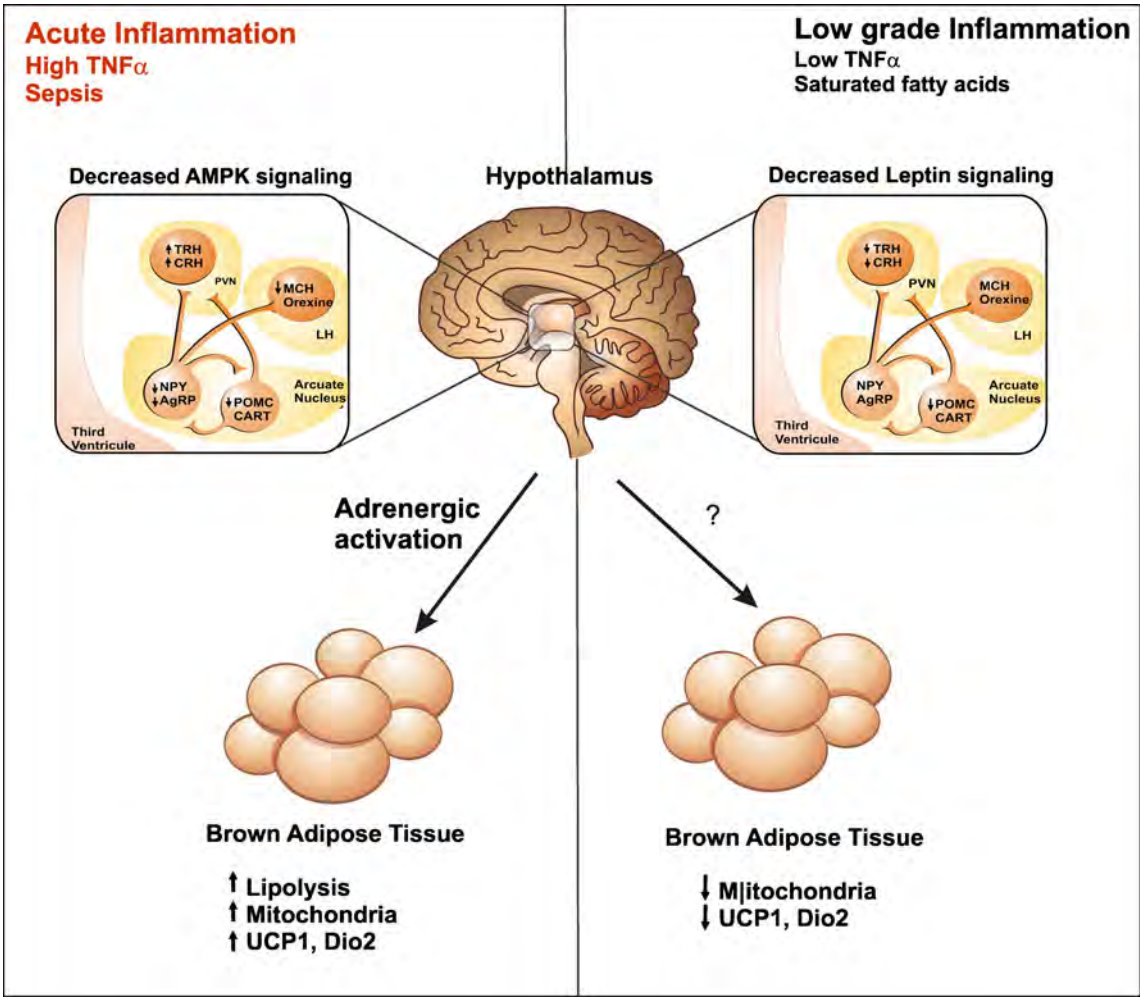

Fig. 2 Different outcomes of low- and high-grade inflammation of the hypothalamus. In high-grade inflammation (left-hand side) increased expressions of TRH and CRH occur, accompanied by reduced expressions of MCH, NPY, AgRP and POMC. Additionally, increased BAT thermogenic activity, driven by adrenergic inputs is observed. In low-grade inflammation (right-hand side) increased expressions of TRH and CRH are seen with a reduced expression of POMC; reduced BAT thermogenic activity is driven though a yet unknown mechanism

a saturated fatty acid able to induce hypothalamic inflammation (i.e., TNF-α increase) (Milanski et al. 2009), also reduced the expression of UCP1 in BAT. Although we have not explored the mechanisms that effectively deliver the neural signal to the affected peripheral organs, it is tempting to propose that the modulation of the sympathetic tonus could be involved since these animals presented significant leptin resistance and leptin is important for adrenergic activation.

In line with these observations, mice knocked-out for TNFR1, the main TNF-α receptor, are resistant to the obesity induced by high-fat diet by means of increased BAT activation and thermogeneis (Romanatto et al. 2009). Although these animals are whole body knockouts, which means that these effects could be a consequence of TNF-α in tissues other than the hypothalamus, the fact that the inhibition of TNF-α action specifically in hypothalamus in obese animals (icv injection of infliximab) increases energy expenditure strongly suggests that the TNFR1 KO animal phenotype could be at least in part a consequence of the absence of the action of TNF-α in the hypothalamus.

Concluding remarks

Hypothalamic inflammation emerges as an important player in the pathogenetic mechanisms contributing to the development of obesity. The dysfunction generated by this inflammatory process leads to changes in neurotransmitter expression and eventually to neuronal loss.

Both caloric intake and thermogenesis become affected by hypothalamic dysfunction. In animal models of obesity, changes in BAT activity contribute to the overall impairment of thermogenesis and this is, at least in part, due to the

affection of the hypothalamus. Recent studies have provided strong evidence for BAT activity in adult humans (Cypess et al. 2009; van Marken Lichtenbelt et al. 2009; Virtanen et al. 2009). This activity is reduced in obese and elder subjects. Although we have no experimental evidence regarding the dysfunction of the hypothalamus in obese subjects, it will be exciting to develop methods to simultaneously evaluate hypothalamic and BAT function in obesity, and try to establish a functional link between both these tissues.

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