CHAPTER SEVEN

HYPOTHALAMIC INFLAMMATION AND OBESITY

Eliana P. Araújo,* Márcio A. Torsoni,† and Líacio A. Velloso†

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Abstract

Obesity is one of the most prevalent diseases in the modern world. It results from the progressive loss of balance between food intake and whole body energy expenditure. Recent studies have shown that consumption of fat-rich diets induces hypothalamic inflammation and dysfunction which is characterized by defective response to anorexigenic and thermogenic hormones, such as leptin and insulin, leading to anomalous neurotransmitter production and favoring body mass gain. In this chapter, we present the main recent advances in this rapidly evolving field, focusing on the role of hypothalamic inflammation on the genesis of obesity. © 2010 Elsevier Inc.

I. Introduction

Currently, obesity is one of the most important health problems in the world. It affects more than 300 million humans and according to the WHO these numbers will continue growing during the next 15 years (Kopelman, 2000). Recent epidemiological data on obesity regarding distinct regions of the world can be found at www.iuns.org/features/obesity/tabfig.htm.
Except for some rare types of monogenic defects (Farooqi and O’Rahilly, 2006), obesity occurs because of a complex combination of multiple environmental and genetic factors (Galgani and Ravussin, 2008). The consumption of highly energetic and palatable foods is among the most important epidemiological factors predisposing to this disease (Galgani and Ravussin, 2008). However, not all people exposed to this type of diet develop obesity, and this fact has long intrigued researchers. As currently known, the main reason for the protective phenotype is the intrinsic capacity to maintain the homeostatic control of energy stores in the body (Galgani and Ravussin, 2008). Specialized neurons of the hypothalamus play a central role, connecting the information provided by leptin and insulin, regarding the size of peripheral fat depots, with the mechanisms that regulate hunger and thermogenesis (Velloso et al., 2008). As long as the system is perfectly coupled, changes in energy intake are matched by proportional modifications in energy expenditure. Thus, it is clear that, in order to understand the mechanisms behind most cases of obesity, the phenomena that connect the consumption of highly energetic foods with the loss of energy homeostasis must be deciphered.

A series of recent studies has provided a solid basis to support the hypothesis that in diet-induced obesity, the hypothalamus is targeted by an inflammatory process that leads to a defective regulation of the energy homeostasis (De Souza et al., 2005; Milanski et al., 2009; Wisse and Schwartz, 2009; Yang and Hotamisligil, 2008; Zhang et al., 2008). In this chapter, to discuss this complex mechanism, we start by presenting the physiological role of the hypothalamus in the control of food intake and energy expenditure. Next, we discuss how nutrients can disrupt the correct functioning of this highly specialized organ. Finally, we present the mechanisms involved in diet-induced hypothalamic resistance to adipostatic signals.

II. Hypothalamic Control of Feeding and Energy Expenditure

Two distinct subpopulations of neurons of the arcuate nucleus of the hypothalamus act as the sensors for the energy stores in the body and coordinate a complex network of neurons that, in due course, control the balance of hunger versus satiety, and pro- versus antithermogenesis (Flier and Maratos-Flier, 1998; Schwartz et al., 2000). These first-order neurons are equipped with receptors and intracellular molecular systems capable of detecting subtle or chronic changes in the levels of hormones and nutrients present in the bloodstream (Schwartz et al., 2000). The response to these changes is based on the modulation of the firing rate and of neurotransmitter production and release by specific neuron bodies (Horvath, 2005).
The subpopulations of neurons of the arcuate nucleus are characterized by the neurotransmitters each one produces. One of the subpopulations expresses the orexigenic peptides, NPY and AgRP, while the other expresses the anorexigenic POMC (α-MSH) and CART (Horvath, 2005; Schwartz et al., 2000) (Fig. 7.1). Both subpopulations project to the lateral (LH) and paraventricular (PVN) nuclei of the hypothalamus where they control the functions of second-order neurons. In the PVN, two distinct subpopulations of neurons produce the anorexigenic and prothermogenic neurotransmitters, TRH and CRH (Cone, 2005), while in the LH, two other subpopulations produce the predominantly orexigenic neurotransmitter orexin and the predominantly antithermogenic MCH (Cone, 2005) (Fig. 7.1). During fasting or when body energy stores are depleted, the expressions of NPY and AgRP are induced, while POMC and CART are inhibited. This coordinated response is dependent on the simultaneous sensing of decreased nutrient availability, reduced levels of the adipostatic hormones leptin and insulin, reduced levels of the gut hormones CCK, GLP-1, and GIP, and increased levels of the gastric hormone ghrelin (Badman and Flier, 2005). Active NPY/AgRPergic neurons send inhibitory

Figure 7.1 First-order neurons localized in the arcuate nucleus of the hypothalamus provide the signals that modulate food intake and thermogenesis. During fasting, NPY/AgRP neurons are active and control the function of second-order neurons at the paraventricular nucleus (PVN) (inhibiting CRH and TRH neurons) and at the lateral hypothalamus (LH) (stimulating orexin and MCH neurons). After a meal, or following leptin/insulin stimulation, NPY/AgRP neurons are inhibited and POMC/CART neurons become active, providing the opposite signals.
projections to the PVN, reducing the expressions of TRH and CRH, and stimulatory projections to the LH, boosting the activities of the orexin and MCH expressing neurons. Conversely, following a meal, or when body energy stores are replenished, NPY/AgRPergic neurons are inhibited and POMC/CARTergic neurons are active. In this context, nutrient availability and the levels of leptin and insulin as well as the levels of CCK, GLP-1, and GIP are increased. Conversely, the level of ghrelin is reduced. The result is the inhibition of orexin and MCH neurons in the LH and the activation of the CRH and TRH neurons in the PVN (Badman and Flier, 2005; Cone, 2005; Horvath, 2005).

The mechanisms by which second-order neurons effectively control food intake and energy expenditure are under intense investigation. MCH neurons play an important role in the control of energy expenditure (Cone, 2005; Pereira-da-Silva et al., 2003). Increased expression of this neurotransmitter restrains animal motility and reduces the expression of the mitochondrial uncoupling protein, UCP1, in brown adipose tissue, together leading to a reduction in energy output (Cone, 2005; Pereira-da-Silva et al., 2003). Conversely, the knockout of MCH produces a lean phenotype, due to a combined effect on feeding and thermogenesis (Qu et al., 1996; Shimada et al., 1998), while the knockout of the main MCH receptor, MCHR1, produces a lean phenotype, predominantly due to increased energy expenditure (Marsh et al., 2002). Orexin has a predominant role in arousal and the control of feeding (Chemelli et al., 1999; Farr et al., 2005). Injection of this neurotransmitter in the hypothalamus generates a potent orexigenic stimulus, however, little is known about the mechanisms behind this response (Farr et al., 2005). In fact, recent studies shown that orexin signaling through orexin receptor-2 has an anorexigenic instead of orexigenic action and the enhanced expression of this receptor protects against obesity (Funato et al., 2009). In opposition to the neurotransmitters of the LH, the PVN neurotransmitters, TRH and CRH, have rather overlapping roles in the control of hunger and thermogenesis (Appel et al., 1991; Schuhler et al., 2007). TRH biosynthesis and release is controlled by multiple inputs coming from POMC neurons, leptin direct signals, and from other sources such as T3 (Fekete and Lechan, 2007). Although most studies explore the role of TRH upon the control of thyroid function and, consequently, on thermogenesis, there are plenty of data showing its direct action in the control of feeding (Valassi et al., 2008). Similar to TRH, CRH production is modulated by a number of different inputs, such as signals emanating from the arcuate nucleus and direct actions of leptin, GLP-1, and histamine (Nicholson et al., 2004; Sarkar et al., 2003). The reduction of appetite is the most studied effect of CRH, but several studies point to its prothermogenic effects as well (Solinas et al., 2006). However, little is known about the mechanisms controlling these phenomena. Some additional pathways of the central nervous system play modulatory roles in energy balance. The connections of first- and second-order neurons of the hypothalamus with...
these systems are only beginning to be deciphered (Horvath, 2005). The actions of serotonin and norepinephrine to induce satiety and increase energy expenditure have been known for a long time (Leibowitz and Miller, 1969; Waldhäll et al., 1981). Nevertheless, these neurotransmitters play rather unspecific and minor regulatory roles in this context, as evidenced by the moderate/severe adverse effects produced by drugs acting through the control of these neurotransmitters and by the limited efficiency of all treatment regimens employing such drugs (Mancini and Halpern, 2006).

Recently, a new player came onto the scene. The development of drugs that interact with the receptor for the endogenous cannabinoid system revealed an additional mechanism for the control of food intake and thermogenesis. The first clues about the orexigenic properties of the endogenous cannabinoid system came from the observation that the consumption of exogenous cannabinoids present in marijuana produced a powerful sensation of hunger (Di Marzo and Matias, 2005). The characterization of the main endocannabinoid receptor, CB1, and the development of synthetic antagonists for this receptor created hope for the production of new, safer, and more effective drugs for the treatment of obesity (Di Marzo and Matias, 2005). However, clinical data show that the weight loss produced by this class of drug is only marginally superior to that produced by inhibitors of serotonine reuptake and adverse affects, such as depression and increased rate of suicide, have precluded the widespread use of these compounds for the treatment of obesity (Vinod and Hungund, 2006).

Although, as discussed above, a number of mechanisms play a role in the control of hypothalamic neurons involved in the regulation of energy homeostasis, leptin and insulin are regarded as the most robust peripheral signal providers to the hypothalamus (Schwartz et al., 2000). Leptin is produced predominantly by the adipose tissue in direct proportion to body fat mass (Myers et al., 2008), and although some peripheral actions have been attributed to this hormone, such as the regulation of insulin production by pancreatic β-cells (Seufert et al., 1999), modulation of insulin action (Barzilai et al., 1997), and control of a number of immune functions (Girasol et al., 2009; Mansour et al., 2006; Matarese and La Cava, 2004), the basomedial hypothalamus, particularly the arcuate nucleus, is the main site of its action (Myers et al., 2008; Schwartz et al., 2000).

Both NPY/AgRPergic and POMC/CARTergic neurons express high levels of the main form of the leptin receptor, the ObRb (Myers et al., 2008; Schwartz et al., 2000) (Fig. 7.2). This is a monomeric transmembrane protein that belongs to the type I cytokine receptor family (Munzberg and Myers, 2005; Myers et al., 2008). Like the other members of this family, the ObRb lacks intrinsic enzymatic activity and depends on at least one associated kinase, JAK2, to transduce its signal (Munzberg and Myers, 2005). Upon leptin binding to the ObRb, a dimmerization of receptor units accompanies JAK2 autophosphorylation and the tyrosine phosphorylation
of two residues (Tyr985 and Tyr1138) in the receptor itself (Munzberg and Myers, 2005). These events generate the possibility of activation of at least three distinct intracellular signaling pathways (Munzberg and Myers, 2005). Tyrosine phosphorylation of Tyr985 recruits the tyrosine phosphatase SHP2, which mediates the activation of the p21ras/ERK signaling pathway (Munzberg and Myers, 2005; Myers et al., 2008). Tyrosine phosphorylation of Tyr1138 recruits STAT3 to the ObRb, leading to STAT3 tyrosine phosphorylation and translocation to the nucleus, providing a fast-track for leptin-induced control of gene expression (Myers et al., 2008). Finally, the autophosphorylation of JAK2 leads to the recruitment and tyrosine phosphorylation of adaptor proteins IRS1/2, which promotes the activation of PI3-kinase and its downstream signaling (Myers et al., 2008). It is possible that several other signaling pathways are also activated through the ObRb, these may include other substrates for JAK2, since a large number of Tyr residues may be phosphorylated following kinase activation, and the engagement of as yet unknown tyrosine kinases, since signal transduction through IRS1/PI3-kinase/Akt can occur even in the absence of the activation of JAK2 (Mansour et al., 2006).

**Figure 7.2** Leptin (ObRb) and insulin receptors transduce the signals generated by their respective ligands though a cascade of signaling molecules of the JAK/STAT and/or IRSs/PI3kinase pathways. So far, five inhibitory mechanisms have been identified that hamper proper signaling through these pathways, SOCS3, PTP1B, JNK, NFκB, and PKCε.
Each of the signaling pathways controlled by leptin plays a role in a specific compartment of the complex response to this hormone. In POMC/CARTergic neurons, the activation of JAK2/STAT3 signaling by leptin leads to increased transcriptional activity, boosting the expression of POMC (Cone, 2005; Schwartz et al., 2000). This effect is enhanced by simultaneous insulin action, but apparently not reproduced by insulin alone (Carvalheira et al., 2001). Conversely, the activation of PI3 kinase activity seems to play a minor role in the control of neurotransmitter expression, but is essential for neurotransmitter release in the synaptic terminals. This effect is achieved through the control of neuronal firing rate. Once activated by either leptin or insulin, PI3 kinase mediates neuronal depolarization by inhibiting ATP-sensitive potassium channels (Horvath, 2005; Niswender et al., 2004). The central role for PI3 kinase in this context is further evidenced by the fact that genetic or pharmacological modulations of phosphatases that control the signaling through PI3 kinase, such as PTEN and 5’-ptase IV, have profound effects on feeding behavior (Bertelli et al., 2006; Plum et al., 2006).

Since not only POMC/CARTergic neurons, but also the orexigenic NPY/AgRPergic neurons harbor the leptin receptor, an important question was raised regarding the mechanisms by which leptin can simultaneously activate the anorexigenic neurons, while inhibiting the orexigenic neurons. The answer to this question came from the demonstration that POMC/CARTergic neurons project inhibitory fibers to NPY/AgRPergic neurons. When leptin levels are high, the activation of POMC/CARTergic neurons leads to a simultaneous inhibition of NPY/AgRPergic neurons, a phenomenon that superimposes the direct signals eventually generated through the ObRb, and also the insulin receptor present in these orexigenic neurons (Horvath, 2005).

Finally, it is important to mention that, besides its predominant actions in the arcuate nucleus, a number of studies have shown that leptin can act through cells present in other regions of the brain. Some of these cells may act only to modulate the main signals delivered by arcuate nucleus neurons, but in some cases specific responses may control other physiological phenomena primarily controlled by leptin (Hayes et al., 2009; Leinninger et al., 2009).

Insulin is the second most important adipostatic signal provider to the hypothalamus. Studies from the late 1960s pioneered the investigation into the roles of insulin in the central nervous system (Margolis and Altszuler, 1967). However, only after the identification of leptin in 1994 were the functions of insulin in the hypothalamus described (Air et al., 2002; Carvalheira et al., 2001). In the arcuate nucleus, both NPY/AgRPergic and POMC/CARTergic neurons express receptors for insulin (Schwartz et al., 2000; Velloso et al., 2008). As in peripheral tissues, in the hypothalamus, insulin activates signal transduction through IRS1 and IRS2, leading to the engagement of the
PI3K/Akt/Foxo1 pathway (Carvalheira et al., 2001; Schwartz et al., 2000; Torsoni et al., 2003). In addition, insulin induces a potent cross-talk with the leptin signaling pathway through the activation of JAK2 (Carvalheira et al., 2001). In fact, one of the most important functions of insulin in the hypothalamus is to enhance leptin’s signal (Carvalheira et al., 2001; Schwartz et al., 2000). In the absence of the insulin signal in the hypothalamus, much of the adipostatic function of leptin is lost, as demonstrated using neuron-specific insulin receptor knockout mice (Bruning et al., 2000).

Therefore, under physiological conditions the hypothalamus, acting under the control of peripheral factors, coordinates perfect coupling between food intake and energy expenditure. As long as the system is fully active, body mass is maintained steady.

III. NUTRIENT-INDUCED DYSFUNCTION OF THE HYPOTHALAMUS

Excessive caloric intake, regardless of the type of nutrient consumed, is a primary risk factor for the development of obesity (Cohen, 2008). However, a number of epidemiological studies have shown that populations consuming preferentially diets rich in fat are especially prone to gain body mass (Damiao et al., 2006; Ogden et al., 2007). In addition to its caloric value, fatty acids are known to exert functional modulation of several tissues and cell types. Therefore, we tested the hypothesis that high fat consumption could modulate gene expression in the hypothalamus. Using a macroarray approach, the expressions of more than 1000 hypothalamic genes were simultaneously evaluated. More than 15% of the analyzed genes were somehow modulated by the diet. Clustering the genes by function revealed that immune response related genes were the most affected (De Souza et al., 2005, 2008). Among the fatty acids commonly present in the occidental diet, the long-chain saturated ones are the most harmful. By activating signal transduction through receptors of the toll-like receptor family, especially the TLR4/MyD88, these fatty acids activate an inflammatory response by the microglia cells in the hypothalamus (Kleinridders et al., 2009; Milanski et al., 2009). Signaling through JNK and NFkB leads to the induction of cytokine gene transcription and local levels of TNF-α, IL-1β, IL-6, and IFN-γ rise and nourish hypothalamic inflammation (Milanski et al., 2009).

Following fatty acid-induced activation of TLR4 signaling, cells turn on an adaptive mechanism that has the biological purpose of adapting protein synthesis to the harms imposed by the inflammation. This mechanism is called endoplasmic reticulum stress and, depending on the magnitude and duration of the harm, can enhance inflammatory signal or induce apoptosis (Milanski et al., 2009; Zhang and Kaufman, 2008).
The ER is the organelle responsible for the synthesis and processing of membrane and secretory proteins (Xu et al., 2005). When the ER homeostasis is disrupted, the accumulation of misfolded and unfolded proteins in the ER lumen ensues (Schroder and Kaufman, 2005; Xu et al., 2005). To deal with this condition, the affected cells activate a complex signaling system known as the unfolded protein response (UPR), aimed at preserving cell integrity, while the harmful condition persists (Schroder and Kaufman, 2005; Xu et al., 2005). One of the outcomes of the activation of UPR is the induction of the expression of cytokines and proteins involved in immune surveillance (Krappmann et al., 2004; Marciniak and Ron, 2006).

If the exposure to saturated fatty acids is prolonged and, depending on some as yet unknown genetic determinants, a proapototic response is induced, leading to the preferential loss of anorexigenic neurons in the arcuate nucleus (Moraes et al., 2009). As time passes, a gradual modification in the relative numbers of orexigenic and anorexigenic neurons takes place and a novel set point for body adiposity is generated. This fact may explain why some obese patients are so resistant to different behavioral and pharmacological approaches employed to treat obesity. Thus, activation of TLR4 signal transduction, followed by the induction of hypothalamic ER-stress, is the main mechanism linking the high consumption of dietary fats to the induction of hypothalamic dysfunction in obesity.

IV. Hypothalamic Resistance to Anorexigenic Signals

As long as a perfect coupling between caloric intake and energy expenditure is preserved, body adiposity is maintained at a physiological level (Cohen, 2008). One of the mechanisms involved in the breakdown of this equilibrium is the installation of resistance to the anorexigenic and thermogenic effects of leptin and insulin (De Souza et al., 2005). In animal models, the resistance to both these hormones can be quantified by a simple method. In intracerebroventricular cannulated lean rodents, the acute injection of leptin leads to a reduction of up to 60% of spontaneous food intake over 12 h (Carvalheira et al., 2001). Injecting insulin, rather than leptin, produces a reduction of up to 50% in food intake. However, if the same tests are performed in obese animals, the effects of both hormones are blunted by at least 50%. To identify the molecular mechanism responsible for producing the functional resistance to leptin and insulin, several groups have evaluated different models of obesity and the most remarkable findings reveal that, upon diet-induced obesity, the induction of inflammatory activity, specifically in the hypothalamus, leads to the activation of intracellular signaling pathways that promote a negative cross-talk with the leptin
and insulin signaling systems, therefore, impairing their physiological anorexigenic activities (Carvalheira et al., 2001; Torsoni et al., 2003).

Currently, five distinct mechanisms are known to play a role in diet- and cytokine-induced resistance to leptin and/or insulin in the hypothalamus of rodents. Both TNF-α and the consumption of fat-rich diets can induce the activation of the serine-kinase JNK in hypothalamic cells. Activated JNK targets IRS1, catalyzing its serine phosphorylation and hampering its capacity to act appropriately as a docking protein for PI3-kinase. This results in reduced insulin-induced activation of Akt and coincides with functional resistance to insulin. Inhibition of JNK by a specific chemical inhibitor or inhibition of TNF-α by a blocking monoclonal antibody reverses the effects of the diet or the cytokine and reestablishes a normal tonus for insulin activity in the hypothalamus (De Souza et al., 2005).

IKK, the serine kinase involved in IκB phosphorylation/degradation, which leads to NFκB activation, is another intermediary involved in diet-induced insulin resistance in the hypothalamus (Zhang et al., 2008). In rats fed on a fat-rich diet, activated IKK promotes the serine phosphorylation of IRS1, hampering the signal transduction of insulin. While in peripheral organs of insulin resistant animals, the inhibition of IKK by salicylates was proved to reinstall correct insulin activity (Prattali et al., 2005), no data regarding the pharmacological inhibition of this enzyme in the hypothalamus are available so far, but it is expected to work in a similar manner.

SOCS3 belongs to a family of inducible proteins that respond to stimulus by a number of cytokines, hormones, and growth factors. Once expressed, SOCS proteins provide a negative feedback to the original signal, acting as a regulatory loop to restrain over stimulation. In diet-induced obesity, the expression of SOCS3 is significantly stimulated in the hypothalamus, providing a negative control for leptin and insulin signaling (Bjorbaek et al., 1998; Munzberg et al., 2004). At least two mechanisms are involved in SOCS3 inhibition of leptin and insulin signaling. The first depends on the physical interaction of SOCS3 with either the ObRb or STAT3 (Myers et al., 2008). Under these circumstances, the transduction of the signal is inhibited because the sites for protein–protein interaction are blocked by SOCS3. The second mechanism depends on SOCS3-dependent ubiquitination of IRS proteins. Following ubiquitin tagging, IRS proteins are directed to proteosomal degradation, restraining signal transduction through this pathway (Myers et al., 2008). The important role for SOCS3 in diet-induced obesity is further confirmed by the fact that genetic abrogation of SOCS3 is capable of protecting mice from diet-induced obesity (Myers et al., 2008).

An additional mechanism involved in diet- and cytokine-induced resistance to anorexigenic signaling in the hypothalamus is the induction of expression of the tyrosine phosphatase PTP1B. Once induced, this enzyme catalyzes the dephosphorylation of the IR and IRS proteins, turning off the signals generated by insulin. The knockout of the PTP1B gene or the
knockdown of PTP1B by antisense oligonucleotides protects experimental animals from diet- and cytokine-induced insulin resistance in the hypothalamus (Picardi et al., 2008).

Finally, a recent study showed that consumption of saturated fatty acid-rich diet promotes the activation of PKC-θ in discrete neuronal populations of the arcuate nucleus leading to insulin and leptin resistance in the hypothalamus (Benoit et al., 2009).

V. Concluding Remarks

Following the identification of leptin, 15 years ago, great advance has been obtained in the understanding the physiological mechanisms of body mass control. This has allowed substantial advance in the characterization of the mechanisms involved in the development of obesity. It is currently believed that, in most cases of obesity, the hypothalamus is the primarily affected organ. Upon high consumption of dietary fat a local inflammatory process is triggered by the activation of TLR4 signaling. Neurons and microglia are affected and ER-stress is induced. Depending on genetic background, specific subpopulations of neurons are lost by apoptosis, therefore enhancing the harmful effects of inflammation. With time, the homeostatic control of body energy stores is lost and obesity emerges. Future studies will focus on the characterization of similar phenomena in human beings and on the evaluation of specific antiinflammatory approaches to treat obesity.

ACKNOWLEDGMENTS

Studies performed at the Laboratory of Cell Signaling were supported by grants from Fundação de Amparo à Pesquisa do Estado de São Paulo, Conselho Nacional de Desenvolvimento Científico e Tecnológico. The Laboratory of Cell Signaling is a member of the National Institute of Obesity and Diabetes (INCT de obesidade e diabetes—CNPq/MCT). The author thanks Dr. N. Conran for editing the English grammar.

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