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Abstract

One important lesson from the last decade of studies in the field of systemic energy metabolism is that obesity is first and foremost a brain disease. Hypothalamic neurons dysfunction observed in response to chronic metabolic stress is a key pathogenic node linking consumption of hypercaloric diets with body weight gain and associated metabolic sequelae. A key hypothalamic neuronal population expressing the neuropeptide Pro-opio-melanocortin (POMC) displays altered electrical activity and dysregulated neuropeptides production capacity after long-term feeding with hypercaloric diets. However, whether such neuronal dysfunction represents a consequence or a mechanism of disease, remains a subject of debate. Here, we will review and highlight emerging pathogenic mechanisms that explain why POMC neurons undergo dysfunctional activity in response to caloric overload, and critically address whether these mechanisms may be causally implicated in the physiopathology of obesity and of its associated co-morbidities.

Abbreviations:

POMC: Pro-opio-melanocortin, NPY: neuropeptide Y, AgRP: agouti-related protein, MCRs: melanocortin receptors, ARC: arcuate nucleus of the hypothalamus, α-MSH: α-melanocyte stimulating hormone, GABA: γ-Aminobutyric acid, LpRB: leptin receptor, IR: insulin receptor, PTP1B: protein tyrosine phosphatase 1B, SOCS3: suppressor of cytokine signaling 3, PI3K: phosphoinositide 3-kinase, TCPTP : T-cell protein tyrosine phosphatase, IKKβ: Iκβ kinase, ROS: reactive oxygen species, ER: endoplasmic reticulum, TLR4: toll like receptor 4, mTOR: mechanistic target of rapamycin, DRP1: dynamin related protein 1, JNK:

Keywords:

hypothalamus; obesity; diet; POMC neurons; electrical activity

The hypothalamic melanocortin system and its role in the regulation of energy balance

Our body undergoes continuous changes in energy availability that must be finely tuned so to guarantee survival. Energy homeostasis involves an unceasing process of communication between the brain and peripheral organs, which ensures constant levels of energy reserves, aiming at maintaining a stable body weight throughout life. When energy availability is increased, such as during a meal, hormones and metabolites produced by peripheral organs inform specialized neuronal circuits that are in turn able to reduce appetite and to concomitantly increase energy expenditure, so to defend the body weight (Schwartz et al., 2000; Clemmensen et al., 2017). In presence of low energy reserves (such as in fasting), these circuits are instead engaged to stimulate the search and intake of food and to reduce energy dissipation, in order to respond to the body's energy demands (Aponte et al., 2011; Krashes et al., 2011). These short-term mechanisms of neuronal adaptation are associated with the long-term metabolic control exerted by hormones such as leptin and insulin [amongst others (Woods et al., 1998; Cota et al., 2007)], that adjust energy balance-related mechanisms in response to prolonged changes in dietary habits or physical activity. The relevant role played by the hypothalamus in this context has been known since decades, thanks to initial observations made using experimental animal models of hypothalamic lesions (Olney, 1969; Leibowitz et al., 1981; Bergen et al., 1998). The ablation of different hypothalamic areas provoked an altered response to both short-term and long-term changes in the energy status (Morton et al., 2014). It took several years to identify the major neuronal circuits involved, albeit it was clear already from these initial studies that some of these circuits relevant for energy balance were likely located in the arcuate nucleus of the hypothalamus (ARC) (Olney, 1969; Hamilton et al., 1976). The key role of ARC neurons expressing the pro-hormone pro-opio-melanocortin (POMC) emerged thanks to some serendipitous discoveries. While it was clear that pro-opio-melanocortin, the main protein product of POMC neurons, gives rise to different posttranslational products (named melanocortins) implicated in skin pigmentation (Gantz and Fong, 2003; Anderson et al., 2016), the metabolic implications of melanocortins and of their 5 receptors (referred to as melanocortin receptors, MC1R-MC5R) were initially unknown. Due to the expression profile of MC4R at the level of neuronal circuits known to affect

neuroendocrine and autonomic processes (Mountjoy et al., 1994), its likely role in the control of energy balance was hypothesized. However, a clearer understanding of MC4R physiology came only from a breakthrough discovery, which was achieved while trying to decipher the function of another melanocortin receptor, MC1R (Lu et al., 1994). Agouti is a 132-amino acid protein produced by the hair follicle. This protein was known to have a high affinity for MC1R, and to competitively block the binding of the melanocortin α-MSH at the level of this specific receptor (Lu et al., 1994). Strikingly, Lu and colleagues observed that agouti could also behave as a competitive blocker of α-MSH binding to MC4R (Lu et al., 1994). In this same period, mutations in the agouti genes leading to abnormal (ectopic) expression of this protein were identified in a mouse model carrying an obese phenotype (*Ay* yellow mice) (Yen et al., 1994; Klebig et al., 1995). This observation led to the hypothesis that the phenotype could originate from the inhibition of melanocortin receptor(s) outside the hair follicle (Lu et al., 1994). The central role played by MC4R in energy balance regulation was soon after confirmed by a serious of observations: i) murine models with genetic impairment of MC4R were morbidly obese (Fan et al., 1997; Huszar et al., 1997), and ii) pharmacological administration of a α-MSH analogue in the brain suppressed food intake in three different mouse models (fasted C57BL/6J, leptin-deficient *ob/ob,* and *Ay* mice), while this effect was blocked by co-injection of pharmacological antagonists of MC3R and MC4R (Hruby et al., 1995; Fan et al., 1997). These findings collectively supported the idea that hypothalamic POMC expressing neurons release α-MSH to inhibit feeding via activation of MC4R's located in the brain, and that chronic blockade of this pathway by agouti ultimately drives the obese phenotype observed in *Ay* yellow mice. The subsequent discovery and characterization of the agouti-related protein (AgRP) (Fong et al., 1997; Graham et al., 1997; Ollmann et al., 1997) has then provided an attractive final functional mode of action for POMC neurons. Neuropeptide Y (NPY)/ AgRP-producing neurons were recognized as a distinct local counterpart of the proopiomelanocortin (POMC) cells (Hahn et al., 1998), and it became soon clear that, like agouti, AgRP acts as competitive antagonist of α-MSH at the level of MC4R (Ollmann et al., 1997).

After more than 20 years of seminal research on this topic, we now know that NPY/AgRP neurons and POMC neurons by means of oppositely controlling MC4R signalling are considered the 'Yin and Yan' of feeding regulation. When POMC neurons are activated, this leads to the release of α-MSH from the axon terminals,

which activates MC4Rs on target neurons, leading to suppression of food intake and to increased energy expenditure (Balthasar et al., 2005). By contrast, when the activity of NPY/AgRP neurons is increased, AgRP is released and antagonizes the effect of α-MSH produced by POMC neurons on MC4R (Ollmann et al., 1997), thereby increasing food intake. The NPY/AgRP neurons not only antagonize melanocortin neurons at their target sites where MC4Rs are located, but also directly inhibits POMC perikarya (Tong et al., 2008). Such direct inhibition, which involves NPY and the small inhibitory amino acid neurotransmitter γ-Aminobutyric acid (GABA) (Cowley et al., 2001; Tong et al., 2008; Atasoy et al., 2012), lowers the activity of POMC cells whenever the NPY/AgRP neurons are active (Tong et al., 2008). The activity of melanocortin neurons is dynamically fine-tuned by a wide range of nutrient and hormonal signals (Schwartz and Porte, 2005; Toda et al., 2017), whose function is to guarantee the body's energy homeostasis. In this context, the hormone leptin plays a central role. This hormone is predominantly made by adipose cells to inhibit hunger and it acts as an adiposity signal informing the brain about the availability of stored energy in the form of fat (Woods and Seeley, 2000). The ARC, and the melanocortin circuit specifically, is a primary site of action for the satiety and the metabolic effects of leptin (Satoh et al., 1997; Cowley et al., 2001; Xu et al., 2018). Of note, we are learning from more recent studies that POMC neurons activity is not only influenced by circulating messengers in the blood-stream. Indeed, sensory detection of food suffices to increase POMC neurons firing rate, while AgRP neurons activity is switched-off (Chen et al., 2015), and these rapid neuronal changes prepare the organism to the imminent ingestion of food (Brandt et al., 2018). Thus, the melanocortin circuit can rapidly and continuously adapt its activity not only in response to internal variations in the body's energy status, but also following sensorial changes triggered by the external world. POMC neurons activity is therefore fundamental for body-weight control as well as for other aspects of systemic energy metabolism that will be further detailed below.

Role of hypothalamic POMC neurons in obesity

Large scale genomic studies have convincingly shown that most of the genetic variations associated with high body mass index involve genes expressed in the central nervous system (Locke et al., 2015). These mutations influence synaptic

transmission and intracellular processing of metabolic signals triggered by nutrients, their metabolites and/or hormones (Locke et al., 2015), particularly at the level of the hypothalamic melanocortin system (Locke et al., 2015; Turcot et al., 2018). Genetic profiling of overtly obese human subjects has revealed that a dysfunctional hypothalamic melanocortin circuit can cause obesity. Homozygous null mutations in the *POMC* gene result in severe weight gain (Krude et al., 1998), while heterozygous loss-of-function affecting the coding for α- and β-MSH significantly increase the risk of metabolic disease (Biebermann et al., 2006; Lee et al., 2006). Genetic disruption of *Mc4r* in mice leads to hyperphagia and increased fat mass (Huszar et al., 1997), a phenotype that overlaps entirely with that seen in humans with MC4R mutations (Farooqi et al., 2003). Although these studies clearly show that impaired POMC neurons function provokes increased food intake and consequently obesity, the phenotypical changes observed in response to deletion of the *POMC* gene or of MCRs may not fully reveal the whole span of metabolic effects controlled by these neurons. Recent approaches whereby POMC neurons function has been globally impaired (Quarta et al., 2019) or completely lost (Gropp et al., 2005; Greenman et al., 2013; Zhan et al., 2013) have provided more comprehensive information. These studies have shown that global loss-of-hypothalamic POMC neurons activity leads to obesity, not only by causing hyperphargia (Greenman et al., 2013), but also by reducing energy expenditure (Greenman et al., 2013; Quarta et al., 2019), due to lowered activity of the peripheral sympathetic nervous system and consequent reduced thermogenic activity of the adipose tissues (Greenman et al., 2013). Accordingly, obese subjects heterozygous for MC4R display signs of decreased vasoconstrictive muscle sympathetic nerve activity, a direct measure of central sympathetic nervous outflow (Sayk et al., 2010). We have recently observed that loss of the transcription factor T-Box3 (Tbx3) specifically in POMC neurons (POMC-Tbx3-Ko) provokes impaired energy expenditure and obesity as a result of a ‘crisis’ of neuronal identity, which makes these neurons unable to express their main neuropeptidergic markers (Quarta et al., 2019). Thus, one take-home massage from these loss-of-function studies is that ARC POMC neurons are not only essential for controlling feeding, but they can also impact the body’s metabolic efficacy, by coordinating the activity of peripheral organs, via the sympathetic nervous system. A second important aspect emerging from these loss-of-function studies is that impaired POMC neurons function, or POMC neurons ablation, leads to a broad

spectrum of metabolic disorders known to be associated with human obesity, including higher cholesterol levels (Greenman et al., 2013; Zhan et al., 2013), glucose intolerance (Greenman et al., 2013; Zhan et al., 2013; Quarta et al., 2019), and insulin resistance (Greenman et al., 2013), thus suggesting that POMC neurons dysfunction may negatively impact metabolic health beyond increased feeding and elevated body weight. This possibility is supported by several pieces of evidence: i) pharmacological inhibition of MC4R activity in the hypothalamus increases plasma cholesterol in mice, without inducing changes in food intake or body weight (Perez-Tilve et al., 2010); ii) mice lacking serotonin 2C receptors in POMC neurons display systemic insulin resistance as a result of an impaired insulin action in the liver, independent from changes in body weight or fat mass (Xu et al., 2010); iii) genetically-induced loss of leptin action in POMC neurons leads to clear-cut changes in systemic glucose control and hepatic insulin sensitivity, without affecting body weight (Berglund et al., 2012). Thus, POMC neurons activity is directly implicated in systemic glucose and lipid metabolism, while a dysfunctional activity of these neurons does not only cause weight gain, but also leads to frank metabolic disease. For all these reasons, anti-obesity drug development strategies in the last decades have focused on fine-tuning the activity of melanocortin neurons (Kühnen et al., 2019). The first generation of MC4R agonists has been tested as anti-obesity agents in patients with stop mutations for the *POMC* gene or with leptin receptor deficiency, but this approach failed, primarily due to safety issues, as these small molecules led to increased blood pressure (van der Klaauw and Farooqi, 2015). Promisingly, a new generation of MC4R agonists has been recently shown to reduce body weight and fat mass in genetically prone obese subjects, without producing cardiovascular adverse events (Kühnen et al., 2016, 2019). Whether POMC neurons dysfunction may critically contribute to dietary-induced obesity, and therefore whether the activity of these neurons could be manipulated so to counteract the global obesity epidemic remains a compelling question. In the next chapters, we will explore the emerging pathogenic mechanisms linking dysregulated POMC neurons activity with the aetiology of diet-induced obesity.

Impact of hypercaloric diets on POMC neurons activity

Since the generation of the first reporter mouse model allowing direct recording of POMC neurons' electrical activity (Cowley et al., 2001), and the demonstration that

leptin excites these neurons (Cowley et al., 2001), the field has comprehensively illustrated that POMC neurons activity is highly sensitive to a plethora of metabolic-related parameters, such as nutrients (glucose, amino acids, free fatty acids), hormones (leptin, insulin, ghrelin, adiponectin, glucagon-like peptide 1, estrogen), cytokines, neuromodulators (serotonin, dopamine, dynorphin) or gliotransmitters (acetyl-CoA binding protein) [recently reviewed in (Zeltser et al., 2012; Dietrich and Horvath, 2013; Toda et al., 2017)]. The different sensitivities to hormones and nutrients would make the melanocortin network highly vulnerable to changes in diet consumption. Accordingly, diet-induced obesity undermines the ability of POMC neurons to appropriately respond to nutrients and hormones, and chronic exposure to high-fat feeding decreases the basal firing rate and excitability of POMC neurons (Paeger et al., 2019), likely due to the convergence of multiple intracellular and intercellular pathogenic alterations (see next section). The electrical activity of POMC neurons largely depends on the synaptic inputs that these neurons receive. In addition to being regulated by classical neurotransmitters such as GABA and glutamate, they can be activated by serotonin (Sohn et al., 2011; Gao et al., 2017) and inhibited by dopamine (Zhang and van den Pol, 2016) or dynorphin (Zhang and van den Pol, 2013; Pennock and Hentges, 2014). The expression of the receptors for these neuromodulators and the response of POMC neurons to serotonin is altered by diet-induced obesity (Romanova et al., 2018). Changes in both glutamatergic and GABAergic synaptic inputs onto POMC neurons have been observed in response to long-term high-fat diet feeding (Klöckener et al., 2011; Newton et al., 2013), and these changes are associated with obesity and altered glucose homeostasis (Klöckener et al., 2011; Newton et al., 2013). Synaptic inputs onto POMC neurons can also rapidly change upon short-term feeding with hypercaloric diets. Three days of overfeeding with a fat-enriched diet increases excitatory post-synaptic currents frequency onto POMC neurons through mechanisms of synaptic remodelling, which are dependent on the level of polysialilation of the adhesion protein neural cell-adhesion molecule (NCAM) (Benani et al., 2012). The hypothalamic mRNA expression of POMC is elevated after 2 weeks of hypercaloric diet feeding in C57BL/6J mice (Ziotopoulou et al., 2000). Thus, it might be speculated that POMC neurons are initially rewired and possibly overactivated in response to high-fat feeding as a defense mechanism against the caloric overload, while prolonged metabolic stress ultimately impairs POMC neurons function. However, although one

study reports that chronic exposure to a hypercaloric diet impairs basal POMC neurons firing rate and excitability (Paeger et al., 2019), high-fat diet induced obesity has been also associated with an overall decrease in the inhibitory synapses onto POMC neurons (Horvath et al., 2010). Such apparent controversy might be explained by the different compositions of the hypercaloric diets employed in the different studies, and/or by the different duration of the exposure to the diet. Additional studies are therefore needed to clarify whether specific dietary components directly alter POMC neurons activity irrespective of caloric intake and whether POMC neurons activity undergo dynamic changes in response to hypercaloric diets. Of note, using an outbred model of diet-induced obesity, it has been recently shown that defective regulation of hypothalamic POMC neurons is the earliest marker distinguishing obesity-prone from obesity-resistant mice (Souza et al., 2016), indicating that dynamic changes in POMC neurons activity might directly contribute to the etiology of diet-induced obesity.

Mechanisms underlying POMC neurons dysfunction in diet-induced obesity

A great number of studies in the field of hypothalamic control of systemic metabolism has allowed dissecting the functional and molecular impact of endocrine and metabolic factors, such as leptin, insulin, glucose, endocannabinoids, serotonin, and free-fatty acids, on POMC neurons activity. While these mechanisms and their implication in energy balance regulation have been comprehensively reviewed elsewhere (Dietrich and Horvath, 2013; Joly-Amado et al., 2014; Toda et al., 2017; Cakir and Nillni, 2019), recent advances made may help understanding why POMC neurons display impaired function in response to metabolic stress. In this section, we will specifically review recent literature linking mechanisms altered in diet-induced obesity with POMC neurons dysfunction.

Altered responses to metabolic hormones

The activity of POMC neurons is closely wired to the long-range communication signals that the body uses to coordinate changes in energy availability. Among those metabolic signals, the hormone leptin is known to play a central role (Schwartz et al., 2000; Morton et al., 2014), and to rapidly rewire POMC neurons function (Pinto et al.,

2004; Dietrich and Horvath, 2013) via both pre- and postsynaptic modes of action (Cowley et al., 2001). Perturbations in such “leptin-POMC neurons axis” may be implicated in the metabolic changes induced by hypercaloric diets. Six days exposure to a high-fat diet is sufficient to inhibit the response of ARC neurons to exogenous leptin injection in mice (Münzberg et al., 2004). Similarly, 2 weeks of high-fat diet feeding attenuate exogenous leptin action in both hypothalamic POMC and AgRP neurons (Olofsson et al., 2013). These changes precede the weight gain induced by the diet, implying a potential direct role for such impaired neuronal leptin action in the pathogenesis of obesity. Experimental evidence obtained using transgenic animal models support this view, as ablation of the leptin receptor (LpRB) in POMC neurons, or in a population of GABA-ergic AgRP neurons that critically control POMC neurons activity, provokes exaggerated fat mass accumulation in mice fed with a high-fat diet (Tong et al., 2008; Bell et al., 2014). Accordingly, POMC-specific loss of key molecular components of the leptin pathway, such as the Protein tyrosine phosphatase 1B (PTP1B) (Aberdein et al., 2017), the suppressor of cytokine signaling 3 (SOCS3) (Wang et al., 2019), and phosphoinositide 3-kinases (PI3K) (Al-Qassab et al., 2009), exacerbates obesity and glucose intolerance in response to the consumption of hypercaloric diets (Al-Qassab et al., 2009; Aberdein et al., 2017; Wang et al., 2019). For a more detailed overview of the molecular machinery controlled by leptin ad the level of hypothalamic neurons, please refer to other seminal reviews published on this specific topic (Villanueva and Myers, 2008; Varela and Horvath, 2012; Zhang et al., 2015; Kwon et al., 2016). Thus, impaired cellular leptin action due to caloric overload, or the so-called “leptin resistance”, might link POMC neurons dysfunction with the aetiology of diet-induced obesity. Although it is widely accepted that leptin resistance is a hallmark of obesity and that altered neuronal leptin action might explain why hypercaloric diets promote weight gain and several other metabolic disturbances (Friedman, 2016; Pan and Myers, 2018), a recent study has allowed to re-formulate the mode of action of leptin in obesity. Indeed, pharmacological inhibition of LpRB activity in diet-induced obese mice further increases body weight and food intake in these animals, demonstrating that, contrary to what the field has believed so far, leptin keeps working, even in obesity (Myers, 2015; Ottaway et al., 2015). These key pharmacological findings suggest that, rather than impairing basal endogenous LpRB function, diet-induced obesity more likely oversaturates neuronal LpRB signalling (Myers, 2015), and this eventually leads to

an impaired ability of this receptor to cope with changes in the body's energy status. Fat mass accumulation due to caloric overload produces a constant elevation in circulating leptin levels, which in turn continuously activates neuronal LpRB. Such permanent activation state results into the upregulation of several intracellular negative feedback signals, including the T-cell protein tyrosine phosphatase (TCPTP) (Tiganis, 2013), and SOCS3 (Myers, 2015), which inhibit the maximal signaling capacity of the receptor. Thus, impaired LpRB flexibility contributes to the development and the progression of metabolic disease, simply because this receptor cannot be stimulated to a level that would allow leptin to counteract diet-induced weight gain. Of note, this emerging mode of action of leptin under obesogenic conditions may be also extended to other hormones that impact POMC neurons activity, such as insulin (Spanswick et al., 2000). Leptin and insulin receptors (IR) share several common molecular pathways in hypothalamic POMC neurons (Ruud et al., 2017). Insulin can indeed regulate the activity of POMC neurons by modulating the activity of the tyrosine phosphatase TCPTP (Tiganis, 2013), an enzyme that is not only implicated in the leptin signaling pathway (Dodd et al., 2017), but that also inhibits IR function (Tiganis, 2013). Regulation of IR signaling by TCPTP dictates whether POMC neurons are activated or inhibited by insulin, and this mechanism coordinates hepatic glucose metabolism in response to feeding and fasting (Dodd et al., 2018). Hypothalamic TCPTP levels, along with other known intracellular inhibitors of both leptin and insulin action in POMC neurons, such as PTP1B and SOCS3 are elevated in diet-induced obesity, and these intracellular mechanisms might concomitantly interfere with the maximal activation capacity of both LpRB and IR (Tiganis, 2013). In conclusion, diet-induced obesity undermines the ability of POMC neurons to dynamically respond to changes in multiple circulating metabolic hormones such as leptin and insulin. This molecular impairment has in turn profound negative consequences on metabolic control. Accordingly, the combined genetic ablation of both LpRB and IR in POMC neurons in mice undermines glucose homeostasis and systemic insulin action (Hill et al., 2010), while enhancing the leptin and insulin action via the deletion of the phosphatases PTP1B and TCPTP specifically in POMC neurons reverses diet-induced obesity (Dodd et al., 2015).

Role of inflammation

Diet-induced obesity can lead to a state of systemic low-grade inflammation that is believed to interfere with the functional activity of peripheral organs, and to contribute to the development of numerous comorbidities, including type 2 diabetes, dyslipidemia, and cardiovascular diseases (Gregor and Hotamisligil, 2011). In addition to these peripheral metabolic derangements, inflammatory processes occurring in response to hypercaloric diets can also involve hypothalamic circuits implicated in energy homeostasis (Thaler et al., 2013; Valdearcos et al., 2015; Jais and Brüning, 2017). Activation of key inflammatory mediators such as c-Jun N-terminal kinase (JNK), Iκß kinase (IKKß), and toll like receptor 4 (TLR4) occurs rapidly upon consumption of hypercaloric diets, even prior to significant weight gain (Jais and Brüning, 2017). This results in fostering overeating and in promoting glucose intolerance, as highlighted by the observation that viral or genetic approaches inhibiting the activity of these pathways specifically in the mediobasal hypothalamus, including the ARC, ameliorate the metabolic status of diet-induced obese mice (Zhang et al., 2008; Milanski et al., 2009; Tsaousidou et al., 2014). Using a precision peptide-based molecule able to cell-specifically reverse metabolic inflammation, we have recently observed that this pharmacological approach leads to clear-cut anti-obesity effects in diet-induced obese mice, in part by lowering the hypothalamic inflammatory reaction (Quarta et al., 2017). Exacerbated inflammatory responses occurring in the hypothalamus during exposure to hypercaloric diets contribute to the pathogenesis of diet-induced obesity. Under a mechanistic point of view, this inflammatory reaction likely undermines neuronal activity in a progressive manner. One week of high-fat diet feeding increases the mRNA expression of pro-inflammatory cytokines in the ARC and leads to signs of cellular neuronal injury, particularly at the level of hypothalamic POMC neurons (Thaler et al., 2012), with increased expression of heat shock proteins implicated in protection against cellular stress (Thaler et al., 2012). Of note, proliferation and recruitment of pro-inflammatory microglia in the hypothalamic ARC in response to 3 weeks of HFD is among the first mechanisms favouring weight-gain associated neuroinflammation (André et al., 2017). Accordingly, inhibiting such HFD-induced microglia expansion using an anti-mitotic agent prevents diet-induced hypothalamic inflammation, and blunts diet-induced weight gain (André et al., 2017). With prolonged hypercaloric diet exposure (6-8 months), POMC neurons show increased autophagic activity (Thaler et al., 2012) and signs of mitochondrial stress, due to the chronic local release of pro-

inflammatory cytokines (such as the tumor necrosis factor α, TNFα) from surrounding activated microglial cells (Yi et al., 2017). These long-term neuronal alterations induced by elevated pro-inflammatory signalling can eventually lead to apoptotic events and therefore to POMC neurons death (Moraes et al., 2009; Thaler et al., 2012; Yi et al., 2017; Nyamugenda et al., 2019), albeit not all the studies show signs of POMC neurons loss in response to chronic HFD exposure (Lemus et al., 2015), a controversy that may be explained by differences in dietary composition and length of hypercaloric diet feeding among the studies.

Thus, inflammatory-like reactions dynamically occurring in response to hypercaloric diets might contribute to obesity, at least in part by altering the activity of the hypothalamic melanocortin circuit, and possibly, by inducing POMC neurons cell death. However, several important pieces of this mechanistic puzzle are still missing: how do these inflammatory-like processes culminate into altered POMC neurons activity, and what are the main hypothalamic cellular populations involved? The dynamic of the neuronal inflammatory reaction observed in response to hypercaloric diets closely mirrors the appearance of hormonal inflexibility at the level of POMC neurons. A few days of high-fat diet feeding are sufficient to alter hypothalamic insulin and leptin action in rodents (Clegg et al., 2011; Olofsson et al., 2013). These changes occur concomitantly with the activation of neuronal inflammatory pathways (Jais and Brüning, 2017), such as the IKKβ-NFκB pathway, which, in turn, impacts the activity of molecular responses located at the crossroads between leptin and insulin signalling, such as SOCS3 (Zhang et al., 2008). Brain-specific activation of IKKβ results in increased food intake and body weight gain, likely due to disrupted central insulin and leptin signalling (Zhang et al., 2008). Although definitive mechanistic answers are still awaited, hypothalamic inflammation might undermine POMC neurons activity and ultimately contribute to diet-induced obesity, by interfering with the ability of these neurons to fine-tune their activity in response to changes in the nutrient-related and hormonal milieu. Since hypothalamic astrocytes and microglia can also respond to circulating hormonal signals (such as insulin (García-Cáceres et al., 2016), leptin (Tang et al., 2007; Lafrance et al., 2010; Kim et al., 2014; Gao et al., 2018), and dietary-derived metabolites [such as fatty-acids (Valdearcos et al., 2014)], diet-induced changes in circulating metabolic messengers may similarly interfere with the activity of non-neuronal hypothalamic cells, and this might in turn undermine the hypothalamic melanocortin circuit (Kim et al., 2014). This

possibility is supported by the emerging role of hypothalamic non-neuronal cells in metabolic control (García-Cáceres et al., 2019) and by the observation that hypothalamic glial cells produce gliotransmitters able to influence the activity of the melanocortin circuit (Bouyakdan et al., 2019).

Oxidative stress and altered mitochondrial activity

Obesity is a pathological condition characterized by “metabolic inflexibility”, that is, an impaired ability of the organism to adapt fuel oxidation to fuel availability. Although such inflexibility has been classically explained by alterations in mitochondrial activity in peripheral organs (Turner and Heilbronn, 2008), mitochondrial dysfunction may similarly play a pathogenic role at the level of the brain.

Excessive intake of nutrients provokes an overload of intracellular metabolic substrates (such as fatty acids and glucose) that leads to an increase in the production of Acetyl-CoA. This then causes the over production of NADH through the Krebs cycle, which promotes an increase of electrons with the ability to enter the mitochondrial intermembrane space, ultimately producing an excess of reactive oxygen species (ROS) and oxidative stress (Bournat and Brown, 2010). Within the hypothalamus, nutrient availability directly impacts ROS levels in POMC and AgRP neurons, and, in turn, ROS levels can modify neuronal activity (Andrews et al., 2008; Diano et al., 2011). POMC neurons activity can be rapidly enhanced in response to injections of the ROS H_2O_2 in the brain (Diano et al., 2011), while the application of the H_2O_2 scavenging enzyme catalase during patch-clamp recordings inhibits POMC neurons activity (Pauliina Markkula et al., 2016). A transient increase in ROS levels in POMC neurons favours satiety, while suppression of ROS through intracerebroventricular (icv) administration of the ROS scavenger honokiol promotes food intake, likely due to diminished POMC neurons activity (Diano et al., 2011). Our group has recently demonstrated that the ability of ROS to influence food intake at the level of POMC neurons requires the functional activity of the mechanistic target of rapamycin (mTOR) (Haissaguerre et al., 2018), an intracellular pathway that integrates the actions of nutrients and hormones on food intake (Cota et al., 2006). While a transient increase in ROS levels in hypothalamic POMC neurons might augment their activity and be part of the signalling involved in the physiological regulation of food intake, high ROS level as a consequence of high-fat feeding might instead impair neuronal function, due to alterations in mitochondrial activity (Horvath

et al., 2009). Indeed, at high concentrations, ROS can cause mitochondrial Ca^{2+} release and elevation of Ca^{2+} levels (Barsukova et al., 2011), which may then result in membrane hyperpolarization and in a marked decrease in neuronal excitability (Paeger et al., 2019).

Mitochondrial dynamics and ER stress

Mitochondrial function is intimately linked to the dynamics of these organelles. The morphology and the activity of mitochondria continuously change thanks to processes of fusion and fission between different mitochondria, which typically occur in response to changes in the extracellular environment (Bournat and Brown, 2010; Tilokani et al., 2018). These processes are controlled by fusogenic proteins named mitofusins that impact the connectivity between different mitochondria, and also the physical and functional interaction between mitochondria and other key organelles implicated in metabolic control, such as the endoplasmic reticulum (ER) (Schrepfer and Scorrano, 2016). High-fat diet has been shown to dramatically reduce mitochondria-ER connectivity in POMC neurons, due to an important decrease in the expression of mitofusin 2 (Schneeberger et al., 2013). Loss of mitofusin 2 in POMC neurons alters the mitochondrial shape and, likely due to the decreased tethers with the ER, leads to ER stress, i.e. an impaired ability of this organelle to regulate the synthesis, folding and maturation of intracellular proteins (Schneeberger et al., 2013) (see also section below). These diet-induced intracellular alterations culminate into a defective ability of POMC neurons to process and therefore release α-MSH (Schneeberger et al., 2013). Thus, POMC-specific alterations in mitochondria fusion and, consequently, in ER activity may critically contribute to diet-induced obesity, as also demonstrated by the observation that overexpression of mitofusin 2 in the hypothalamus of diet-induced obese mice ameliorates several metabolic defects in these animals, while POMC selective deletion of mitofusin 1, another mitochondrial fusion protein, causes an obese phenotype (Ramírez et al., 2017). Consistent with this, inducible and selective ablation of the mitochondria fission protein dynamin related protein 1 (DRP1) in POMC neurons increases POMC neuronal activity in response to glucose and leptin and consequently improves glucose metabolism (Santoro et al., 2017) in mice, suggesting that mitochondrial fission regulates the ability of POMC neurons to respond to glucose and leptin. Collectively, these data suggest that mitochondrial dynamics and mitochondria-ER interactions are essential

determinants of POMC neurons dysfunction in response to metabolic stress, and these mechanisms likely impact energy and glucose homeostasis at the systemic level. Changes in the mitochondrial shape induced by metabolic stress may also influence the activity of mitochondria and thus have dramatic effects on intracellular Ca^{2+} handling. Since altered Ca^{2+} homeostasis has been shown to impair POMC neurons excitability in response to a high-fat diet (Paeger et al., 2019), it is reasonable to speculate that altered mitochondria dynamics may also interfere with POMC neurons activity by altering the intracellular Ca^{2+} homeostasis.

Defective proteostasis

Protein folding is a physical process that arranges newly formed proteins into a structural conformation impacting their final biological activity. In mammals, the ER is responsible for folding up most of the membrane and secretory proteins (Wolff et al., 2014), but this elaborate process sometimes generate damaged/misfolded products that can potentially accumulate and undermine cell viability (Ron and Walter, 2007). To minimize the chance of cellular proteins jamming, several systems have evolved to prevent the accumulation of toxic intracellular products. Misfolded proteins can be degraded by the ubiquitin/proteasome system and/or by a mechanism called autophagy, which is the degradation of cellular components by lysosomes or vacuoles. After prolonged feeding with hypercaloric diets, hypothalamic neurons display defective protein homeostasis (or proteostasis) (Cavadas et al., 2016), which may be the result of ER stress (Ozcan et al., 2009; Ramírez and Claret, 2015) and of multiple impairments in intracellular ‘anti-jamming’ systems, such as the ubiquitin/proteasome system (Ignacio-Souza et al., 2014) and autophagy (Meng and Cai, 2011). This leads to aggregation of ubiquitinated and damaged proteins that undermines neuronal activity and negatively impacts body weight control and systemic glucose metabolism. In agreement with this view, mice lacking key autophagy factors in POMC neurons, such as autophagy related 7 and 12 (ATG7 and ATG12), exhibits accelerated weight gain and glucose intolerance, particularly in response to high-fat diet consumption (Coupé et al., 2012; Malhotra et al., 2015). Similarly, loss of key molecular signals involved in ER processing and activity in POMC neurons accelerates the pathogenesis of diet-induced obesity and provokes impaired systemic glucose handling (Yao et al., 2016; Kim et al., 2018). These metabolic phenotypes are associated with impaired *POMC* expression, with

disrupted POMC neurons projections (Coupé et al., 2012; Malhotra et al., 2015; Kim et al., 2018), and, importantly, with an altered ability of POMC neurons to modulate their electrophysiological activity in response to the hormones leptin and insulin (Yao et al., 2016). Additionally, altered hypothalamic proteostasis induced by high-fat diet feeding has been linked with the activation of inflammatory pathways, such as the IKKβ–NFκB and the JNK–AP1 pathways, which in turn contribute to the impaired hypothalamic neuronal activity observed in response to metabolic stress (Zhang et al., 2008; Tsaousidou et al., 2014; Solinas and Becattini, 2016). Thus, defective proteostasis in POMC neurons is tightly linked with molecular mechanisms of inflammation and altered neuronal response to nutrients and nutrient-related endocrine and metabolic factors.

Conclusions

To tackle the growing obesity epidemic and its associated metabolic disorders, there is an urgent need to further decipher the neuronal mechanisms that lead to the dysregulation in energy homeostasis. While decades of research have established that perturbations in POMC neurons function are intimately linked with the aetiology of genetic forms of obesity, recent evidence discussed in this review highlights a potential role for these neurons in the pathophysiology of dietary-induced metabolic disease. Following exposure to hypercaloric diets, several pathogenic mechanisms (**Figure 1**) converge and undermine POMC neurons, independently of diet-induced weight gain, suggesting a causal role for these alterations in the establishment and the progression of the disease. Although most of these mechanistic studies have been performed using murine models, several observations suggest that these pathophysiological nodes might contribute to human obesity. Metabolic inflammation is a hallmark of human obesity (Lumeng and Saltiel, 2011; Hotamisligil, 2017) and signs of ER stress at a systemic level and in peripheral tissues might contribute to the metabolic complications of this disease. Chemical chaperones known to reduce ER stress can also improve β-cell function and insulin resistance at the level of peripheral organs in humans (Kars et al., 2010; Xiao et al., 2011; Cadavez et al., 2014), while a dietary interventions (a hypocaloric diet) in obese patients lead to beneficial metabolic effects that are accompanied by attenuation of systemic

inflammation, and amelioration of ER stress and mitochondrial dysfunction at the level of circulating immune cells (López-Domènech et al., 2019). Whether these same alterations play a contributing role at the level of human hypothalamic neurons remains to be clarified. Nevertheless, the collective evidence hereby reviewed implies that pharmacological interventions able to resolve POMC neurons dysfunction might have promising beneficial effects, a scenario supported by the recent generation of MC4R agonists that safely counteract obesity in subjects carrying POMC or MC4R mutations (Kühnen et al., 2016; Collet et al., 2017), and by the known anti-obesity efficacy of these molecules in preclinical models of diet-induced obesity (Kievit et al., 2013; Clemmensen et al., 2015). However, before conceptualizing novel pharmacological and/or dietary paradigms able to precisely fine-tune POMC neurons function, several key questions still remain. What are the specific molecular underpinnings of such neuronal dysfunction? Can dysregulated POMC neurons activity be reversed, or does long-term overnutrition lead to irreversible changes, such as loss of synapsis (Horvath et al., 2010), cytoarchitectural damage (Horvath et al., 2010), and cell-death (Moraes et al., 2009; Thaler et al., 2012; Nyamugenda et al., 2019), undermining energy balance to a point of no return? And what of the recently described molecular heterogeneity of POMC neurons (Campbell et al., 2017; Chen et al., 2017; Lam et al., 2017)? How does such heterogeneity affect responses to hypercaloric diets? Besides, we should mention that most of the published evidence we have reviewed above has been obtained in male animal models, while obesity is growing at an alarming rate in both sexes (GBD 2015 Obesity Collaborators et al., 2017). POMC neurons activity may have a more profound impact on systemic glucose metabolism in males relative to female mice exposed to hypercaloric diets (Aberdein et al., 2017), and recent evidence suggests the presence of sex dimorphism with respect to POMC neurons injury in response to hypercaloric diets feeding (Nyamugenda et al., 2019). Thus, a better understanding of the dynamic impact of hypercaloric diets on the cellular and molecular machinery of these neurons in both sexes will certainly improve our comprehension of the neuronal pathogenic mechanism underlying obesity and its associated metabolic disorders. Such knowledge may ultimately pave the way to novel and more effective gender-based therapeutics against this disease.

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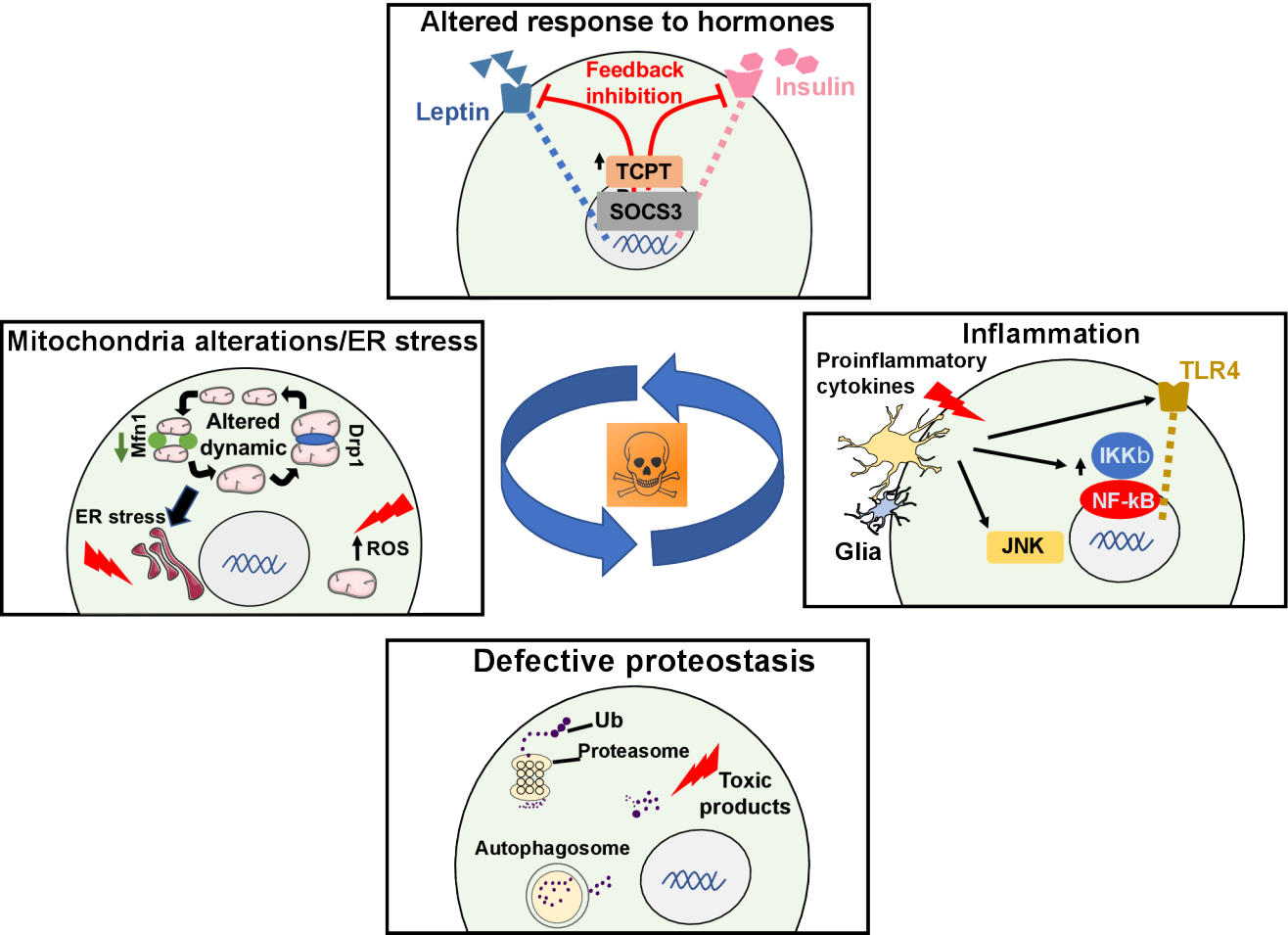
Figures legends

Figure 1: Extracellular and intracellular mechanisms contributing to POMC neurons dysfunction in response to the consumption of hypercaloric diets. TCTP: T-cell protein tyrosine phosphatase, SOCS3: suppressor of cytokine signaling 3, ER:

endoplasmic reticulum, TLR4: toll like receptor 4, MFN1: mitofusin 1, DRP1: dynamin related protein 1, ROS: reactive oxygen species, Ub: Ubiquitin, JNK: c-Jun N-terminal kinase, IKKβ: Iκβ kinase.


Altered response to hormones
Leptin
Feedback inhibition
Insulin
TCPT
SOCS3
Mitochondria alterations/ER stress
Mfn1
Altered dynamic
Drp1
ER stress
ROS
Inflammation
Proinflammatory cytokines
TLR4
IKKb
NF-kB
JNK
Glia
Defective proteostasis
Ub
Proteasome
Toxic products
Autophagosome