Trends in Endocrinology & Metabolism

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Review

Endoplasmic Reticulum Stress, the Hypothalamus, and Energy Balance

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Overweight and obesity pose significant health problems globally, and are causatively linked to metabolic dysregulation. The hypothalamus integrates neural, nutritional, and hormonal cues to regulate homeostasis, including circadian rhythm, body temperature, thirst, food intake, energy expenditure, and glucose metabolism. Hypothalamic neuropeptides play a fundamental role in these processes. Studies during the past two decades suggest a role of central endoplasmic reticulum (ER) stress in the pathophysiology of obesity. This review covers recent findings on the role of ER stress and neuropeptide processing in the central regulation of energy homeostasis, with special emphasis on proopiomelanocortin (POMC)-encoding neurons. In addition, the role of neuroinflammation in the context of obesity is briefly discussed.

Highlights

The obese state induces cellular and metabolic alterations in the hypothalamus, leading to chronic low-grade inflammation and ER stress that, in turn, alters energy balance.

ER membrane-resident sensors transmit ER stress signals to the cell through signaling routes collectively called the unfolded protein response (UPR).

The UPR pathways act in concert to increase ER content, expand ER protein-folding capacity, degrade misfolded proteins, and reduce the load of new proteins entering the ER. All of these are geared toward adaptation to resolve the protein-folding defect.

The hypothalamic ER stress acts to favor a positive energy balance, attenuating the response to the anorectic hormone leptin.

Processing of the hypothalamic POMC is impaired by obesity-induced ER stress, leading to decreased production of α-MSH, a central regulator of energy homeostasis.

Excess Calorie Intake and Neuronal Brain Changes

Food consumption is a fundamental mechanism for survival, and is one of the greatest human pleasures. In modern human society excess eating, in genetically susceptible individuals, is leading to a globally growing obesity epidemic. Among the many metabolic alterations observed in the obese state, central inflammation and ER stress have been of great interest in the recent years because of their impact on energy balance [1].

In contrast to common infection, tissue damage, and stress that can trigger inflammation as a defense mechanism, obesity is manifested as a chronic but relatively low-grade inflammatory state in peripheral tissues as well as in the central nervous system (CNS) [2]. The second phenomenon observed in the obese state, which is the main focus of this review, is ER stress. ER stress is classically triggered by an imbalance between the folding capacity of the ER and the steady-state unfolded protein pool in the lumen. However, there are numerous physiological or pathological triggers of ER stress, including nutrient deprivation, redox status, and ER calcium levels [3]. In addition, several environmental factors such as pollutants including heavy metals, pathogens, physical stress, and various drugs can lead to ER stress and activate the downstream unfolded protein response (UPR) [4]; however, the impact of these factors on central ER stress is unclear, and they will not be further discussed here.

The disturbance in ER homeostasis is communicated to the nucleus and the translational machinery through a set of signaling routes collectively called the UPR (Figure 1). The UPR has three main branches originating with three distinct ER transmembrane proteins that act as ER stress sensors: protein kinase R (PKR)-like ER kinase (PERK; also known as the eukaryotic translation initiation factor 2α kinase 3), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6) (Figure 1). We discuss below some of the recent findings on the role of ER stress and UPR components in the hypothalamic neuronal circuitries that regulate energy balance. We further focus on the interface between ER function and neuropeptide biogenesis, as well as on the role of this interaction in metabolic dysfunction and obesity.

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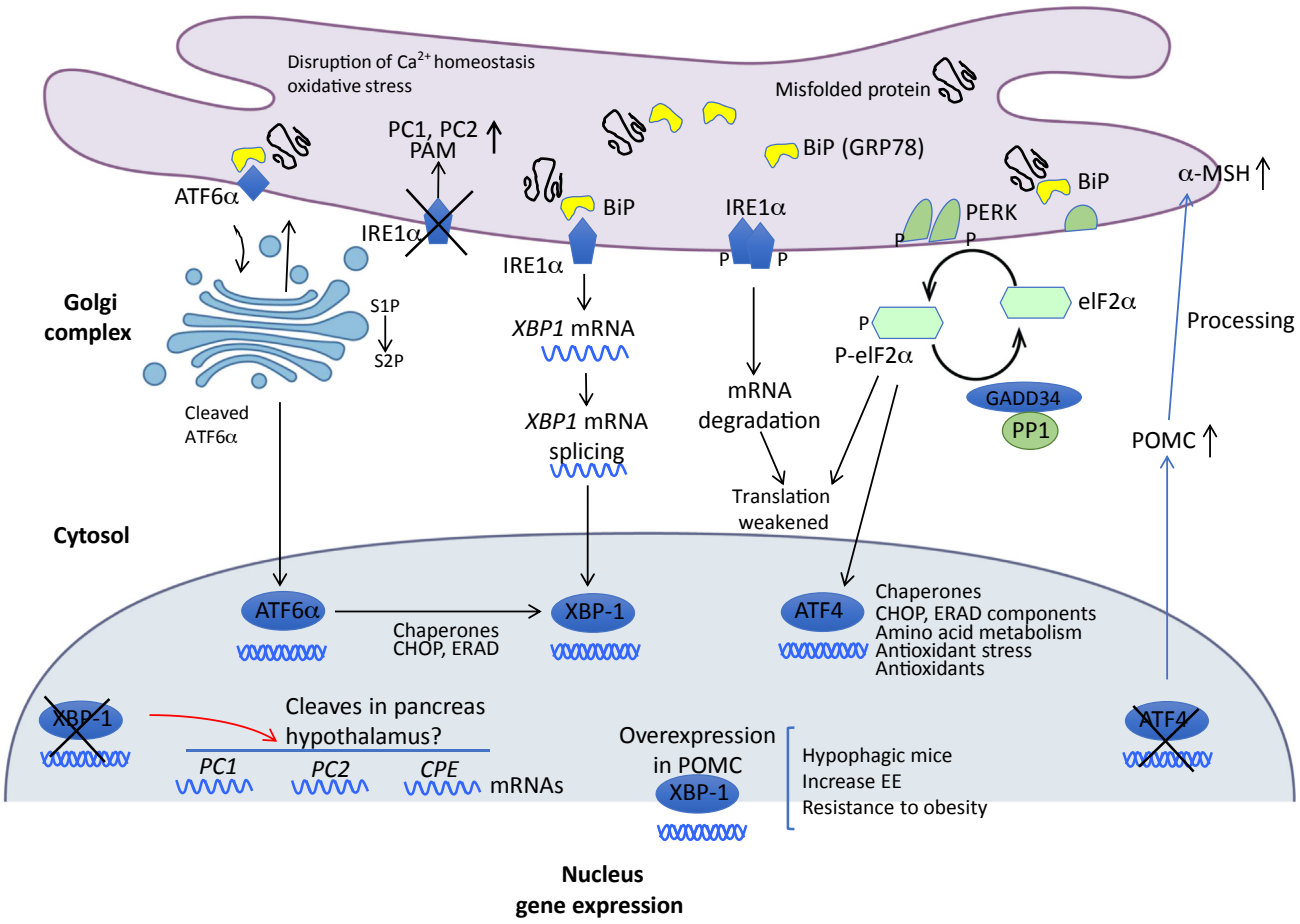

Trends in Endocrinology & Metabolism

Figure 1. The Unfolded Protein Response (UPR). The UPR is composed of three arms that are each initiated by distinct endoplasmic reticulum (ER) membrane proteins: PERK [protein kinase RNA (PKR)-like ER kinase] [56], IRE1 (inositol-requiring protein 1) [57,58], and ATF6 (activating transcription factor 6) [59]. These proteins function as stress sensors that detect the ER protein load and induce adaptive responses aimed to bring the ER folding capacity back within homeostatic boundaries. The excess and accumulation of misfolded proteins in the ER lumen sequesters the chaperone BiP away from the lumenal domain of these ER sensors, leading to their activation. If ER stress cannot be brought back within homeostatic boundaries, prolonged activation of UPR can lead to apoptotic cell death. The lumenal domain of PERK is a stress sensor, whereas its cytosolic domain has enzymatic activity for its substrate eIF2α (eukaryotic initiation factor 2 subunit α). eIF2α phosphorylation (P) leads to global translation attenuation. The other known target of PERK is the transcription factor Nrf2 (nuclear erythroid-related factor 2), the master regulator of the antioxidant response that is involved in the PERK-mediated cell survival in stressed cells [60]. The enzyme(s) that dephosphorylate and inactivate PERK are currently unknown. eIF2α dephosphorylation is regulated by the phosphatase PP1 through its association with either the growth arrest- and DNA damage-inducible protein GADD34 (also called PPP1R15A) [61–63] or PPP1R15B [64]. GADD34 itself is an ER stress-inducible gene acting in a negative feedback loop, whereas PPP1R15B is a constitutive repressor of eIF2α phosphorylation. IRE1 has a lumenal domain, which interacts with GRP78 and can also recognize unfolded proteins, and a cytosolic kinase domain [57,58]. The best-characterized function of IRE1 involves the splicing of the mRNA of a transcription factor (HAC1 in yeast, XBP1 in metazoans) [65], and the IRE1–HAC1/XBP1 arm constitutes the most ancient UPR pathway (Box 1). Dissociation of GRP78 triggers oligomerization of the monomeric IRE1s, but is not sufficient for IRE1 activation. IRE1 oligomers form a luminal surface which allows IRE1 to directly sense unfolded proteins [66], which in turn is thought to trigger a conformational change in the protein, allowing IRE1 dimers to *trans*-autophosphorylate each other. Other than itself, IRE1 does not have any other known substrates as far as its kinase activity is concerned. IRE1 activation is also subject to regulation by other factors. For example, the cytosolic nonreceptor ABL tyrosine kinases localize to the ER membrane during ER stress and potentiate IRE1 RNase activity [67]. ATF6 is an ER transmembrane protein that has a stress-sensing lumenal domain and a cytoplasmic basic leucine zipper domain that acts as a transcription factor upon dissociation from the remainder of the molecule [59]. In unstressed cells ATF6 is bound to GRP78. During ER stress, GRP78 dissociates from ATF6, which triggers ATF6 to localize to the Golgi, where it is processed to release its cytoplasmic DNA-binding domain [68]. ATF6 is processed by site 1 and site 2 proteases in the Golgi in a similar manner to the processing of SREBPs (sterol regulatory element binding proteins) [69,70]. Abbreviations: EE, energy expenditure; α-MSH, α-melanocortin stimulating hormone; PAM, peptidylglycine α-amidating monooxygenase enzyme; PC1 and 2, prohormone convertases 1 and 2; POMC, proopiomelanocortin.

ER and the UPR

The ER is a multifaceted membranous structure extending from the outer nuclear membrane to the cytoplasm, and is involved in several biological processes including protein folding, secretion, lipid biosynthesis, calcium storage, and post-translational modifications such as glycosylation and lipidation. About one third of the human proteome, including secreted, ER-resident, and membrane proteins, transit through the ER. Depending on the tissue type, the representation of secreted and membrane proteins in the total proteome varies greatly. For example, the majority of the pancreatic proteome is composed of secretory proteins, but these constitute only a small percentage of the skeletal muscle proteome. The intracellular expression profile of the chaperones – proteins that assist in the folding/unfolding of other proteins – has evolved to match the nature of the proteome of the corresponding tissue. For example, whereas tissues with higher secreted and/or membrane protein pools, such as pancreas and liver, have higher ER chaperone expression, tissues such as skeletal muscle and skin, where the soluble proteins constitute the majority of the proteome, have a higher expression of cytosolic chaperones. Accordingly, of the >300 proteins in the human chaperone proteome (chaperome), 48 are ER-specific proteins [5]. Together with the ER-associated degradation (ERAD) pathways, the ER chaperones coordinate the folding capacity of the ER to regulate ER protein homeostasis. When the ER protein load exceeds the folding capacity, the UPR is activated to restore ER homeostasis by informing the cytosol and nucleus about the excess protein load, thereby enabling a series of protective mechanisms. Among these responses is the global shutdown of the protein synthesis while specifically activating the translation of chaperone proteins, elevated membrane synthesis to increase ER volume to remodel the secretory apparatus, and degradation of unfolded proteins either through the ubiquitin-proteasome pathway (ER-associated degradation) or in lysosomes (autophagy). Although the UPR can operate to reinstate ER homeostasis, persistent ER stress can also lead to apoptotic cell death [6].

ER Stress and the Regulation of Energy Balance

The regulation of metabolism in mammals requires coordination between tissues and several brain nuclei, and this coordination is mediated by systemic factors and neuronal networks. The past two decades in the metabolism field have uncovered numerous secreted factors that are involved in energy balance regulation, most notably leptin, and dysregulation in the secretion, processing, recognition, or signaling of these factors plays a fundamental role in the etiology of metabolic disorders.

Calorie intake and energy expenditure are also under the control of centrally produced neuropeptide hormones. These neuropeptides are initially synthesized as larger inactive precursors in the rough ER. After their proper folding in the ER, they are then subjected to post-translational modifications in immature secretory granules of the *trans*-Golgi network. Processing continues while secretory granules are vectorially transported to the plasma membrane of the cell ready for secretion [2,3]. The wide abundance, variety, and biological roles of the hypothalamic neuropeptides emphasize the importance of prohormone and neuropeptide processing in the regulation of energy homeostasis, and therefore demands a well-orchestrated ER quality control machinery. The hypothalamus is a heterogeneous population of chemically distinct neurons, with at least 50 distinct cell types in the arcuate nucleus (ARC) and median eminence (ME) alone [7]. Even within defined classes of neuronal populations, such as the agouti related peptide (AgRP) and proopiomelanocortin (POMC) neurons, there are various subtypes, including leptin receptor-positive versus -negative populations. Therefore, the secretory profile of these neuronal populations, and the relative roles of the secretory pathway in the respective neuronal subsets, is likely not uniform.

Alterations in the transcriptional profiles of AgRP and POMC neurons in response to changes in nutritional status highlight the physiological relevance of the UPR in hypothalamic feeding centers. AgRP neurons are typically activated by fasting, which is accompanied by UPR activation in these neurons. Target genes of XBP1s (a transcription factor in the IRE1–XBP1 arm of the UPR; Box 1) including ER chaperones are turned on in the AgRP neurons of fasted animals [8]. The expression of various genes involved in ER protein translocation and Golgi trafficking, as well as transcripts encoding ATF4 and ATF6 (Figure 1), also increase upon food deprivation [8]. Fasting further stimulates the expression of AgRP-specific ERAD-related transcripts. Notably, most of these fasting-induced changes in the UPR pathway are specific to AgRP neurons, and no significant change is detected in POMC cells [8] which produce and secrete the anorectic peptide α-melanocyte stimulating hormone (α-MSH). However, upon short-term refeeding of fasted mice, the UPR in POMC neurons is activated such that the expression of XBP1s, ATF4, and ATF6 increases (Figure 1) [9]. These findings suggest that the UPR in AgRP and POMC neurons is affected differently by nutritional cues, and this correlates with fasting-induced activation of AgRP neurons but suppression of POMC neurons. It is also worth investigating whether fasting-induced changes in AgRP/POMC neurons are functionally coupled to the activity of these vital cell populations.

Several genetic studies have addressed the role of different UPR components in the hypothalamic regulation of energy homeostasis. Although attenuation of the PERK–eIF2α branch of the UPR appears to be protective, the role of hypothalamic IRE1 and ATF6 signaling requires further studies. The PERK–eIF2α branch of the UPR leads to increased translation of ATF4 (Figure 1), a transcription factor that stimulates AgRP expression [10]. Overexpression of ATF4 in the mediobasal hypothalamus (MBH) marginally induces food intake and weight gain, and results in attenuated hepatic insulin signaling, whereas a dominant negative ATF4 improves insulin signaling and glucose metabolism [11]. AgRP ATF4 knockout (KO) mice are protected

Box 1. Inositol-requiring Enzyme-1 (IRE1)

Active IRE1 has endoribonuclease activity towards mRNA encoding X-box binding protein-1 (XBP1) [65,82]. Unprocessed XBP1 encodes a protein that represses UPR genes. Excision of a 26 nt intron results in a frameshift in the XBP1 reading frame. Upon removal of this intron, cleaved *XBP1* mRNA is ligated by RtcB [83], and the resulting shorter mRNA encodes an active UPR transcription factor, XBP1 spliced (XBP1s). *XBP1* transcription is also upregulated by UPR (through ATF6) [82], resulting in increased levels of the unspliced form of XBP1 which serves as an inhibitor of UPR, thereby forming a negative feedback loop [84]. XBP1s is involved in the transcriptional regulation of genes regulating ER biogenesis and ER-associated degradation.

Another important function of IRE1 – that is implicated in linking ER stress to metabolic diseases – involves activation of c-Jun N-terminal kinases (JNK) [85]. Tumor necrosis factor (TNF) receptor-associated factor-2 (TRAF2), an adaptor protein, is recruited by phospho-IRE1 such that TRAF2 associates with the JNK kinases that ultimately result in JNK phosphorylation and activation [85]. The TNF receptor family is known to elicit its activity partly through JNK activation [86], and this pathway depends on intact TRAF2 [87]. JNK can phosphorylate insulin receptor substrate 1 (IRS1) at Ser307, which results in blockade of insulin-mediated IRS1 activation [88].

IRE1 is also engaged in an RNA degradation pathway, called IRE1-dependent decay of mRNA (RIDD) [89], which is not limited to mRNA cleavage and extends to pre-microRNA processing [90]. Substrates for IRE1 RNase activity comprise nuclear, cytosolic, ER, and extracellular targets [89]. IRE1β, one of the two IRE1 isoforms, for example regulates selective degradation of secretory pathway protein mRNAs [91]. XBP1 deletion results in constitutive activation of IRE1 (which is capable of cleaving prohormone convertase 1 and 2) and induction of carboxypeptidase E mRNA in pancreatic β cells [92]. Whether a similar regulation of prohormone processing enzymes exists in hypothalamic neurons is unknown. Likewise, deletion of IRE1α from rat insulin promoter (RIP) positive cells leads to glucose intolerance and attenuates the HFD-induced β cell proliferation commonly observed in obesity, but does not affect body weight or food intake of lean or HFD-induced obese mice. Neuronal XBP1 KOs have comparable body weights to wild-type mice [93,94]. Despite its well-characterized function in the peripheral immune system, the role of XBP1 in microglia and CNS inflammation remains to be uncovered.

from weight gain because of decreased food intake and increased energy expenditure [10]. Deletion of ATF4 from POMC neurons also results in a similar phenotype where mice are resistant to diet-induced weight gain [12,13]. The effect of ATF4 on POMC neurons is largely dependent on negative regulation of autophagy-related protein ATG5 by ATF4. Mice lacking both genes in POMC neurons have reduced energy expenditure on a high-fat diet (HFD) and gain more weight [12,13]. POMC-specific ATG5 KO mice have defective autophagy [14], and deletion of ATF4 results in ATG5-dependent autophagy and increased α-MSH production, leading to increased energy expenditure [12,13] (Figure 1). Whether these findings extend to other orexigenic/anorexigenic neurons remains to be uncovered, however, the above-summarized results collectively indicate that targeting central ATF4 signaling might be a viable strategy to improve overall metabolism and protect from obesity.

The role of the IRE1–XBP1 branch has been studied in the context of POMC neurons. Deletion of XBP1 from POMC neurons does not significantly alter body weight, although the mice show a tendency towards elevated weight gain (S. Mert, Ph.D. thesis, University of Ankara, 2013). Although the KO suppresses caloric intake in lean mice, HFD-fed POMC-specific XBP1 KO mice tend to eat more (S. Mert, Ph.D. thesis). However, the increased food intake of obese mice is compensated by increased energy expenditure, and the obese KO mice have a decreased fat percentage and reduced respiratory exchange ratio, indicative of elevated fat oxidation, than their counterparts (S. Mert, Ph.D. thesis). These results should, however, be evaluated in light of the inherent problems associated with the POMC–Cre line, and the significant difference in the colocalization of POMC–Cre-expressing cells and POMC neurons in adult mice [15]. The outcome of genetic depletion of IRE1α expression in POMC neurons is somewhat controversial: one study reported unaltered body weight on a regular diet, but increased food intake and weight gain with elevated adiposity on a HFD [16]. Obese mice with selective IRE1α KO in POMC neurons also displayed decreased energy expenditure, decreased cold tolerance, and impaired glucose tolerance [16]. These phenotypes could in part be explained by elevated expression of the negative regulators of leptin receptor signaling, including PTP1B and SOCS3 [16]. However, these findings are not supported by another study in which the POMC neuron-specific IRE1 KO mice had increased energy expenditure, and were resistant to HFD-induced weight gain [17]. This latter study concluded that IRE1 depletion led to increased expression of the POMC processing enzymes PC2, CPE, and PAM (Figure 1), leading to increased α-MSH production in the hypothalamus [17]. Overexpression of XBP1s in POMC neurons results in hypophagic mice with increased energy expenditure, and confers resistance to obesity [9] (Figure 1). The positive effect of XBP1 expression in POMC neurons could also be related to the crucial role of this transcription factor in ER biogenesis. In agreement with this hypothesis, genetic manipulations leading to increased ER volume in the hypothalamus can counteract diet-induced adiposity [18].

As summarized above, ATF4 impairs hypothalamic α-MSH processing [12,13]. Therefore, it is worth investigating whether the metabolic improvements in POMC-specific XBP1 transgenic mice are secondary to increased POMC processing and α-MSH production. Given that hypothalamic induction of ER stress attenuates POMC processing, thus leading to decreased α-MSH levels [19] (discussed below), dysregulation of prohormone biosynthesis and processing could be the main routes connecting ER stress to energy metabolism.

ER Stress and Metabolic Dysregulation in Obesity

ER stress and the UPR play a fundamental role in metabolic dysregulation in obesity. Rodent studies suggest that HFD-induced obesity leads to ER stress in multiple peripheral tissues including liver and adipose tissue [20], as well as in the hypothalamus [19,21]. We discuss

below the results from various genetic and pharmacologic studies suggesting that improving ER homeostasis is generally protective against obesity and associated metabolic dysfunction.

Chemical inducers of ER stress, such as homocysteine, tunicamycin, and thapsigargin, increase the expression of negative regulators of leptin receptor signaling including SOCS3 and PTP1B [19,22] in cell lines as well as in hypothalami of lean animals. Homocysteine, a non-protein amino acid that causes protein misfolding, induces XBP1 mRNA splicing in the brain [23],and blocks leptin-induced ERK phosphorylation [22]. Central infusion of thapsigargin, a blocker of the ER calcium importer SERCA (sarcoendoplasmic reticulum calcium transport ATPase), also blocks leptin and insulin signaling in the hypothalamus, and has an orexigenic effect. Furthermore, treatment with tauroursodeoxycholic acid (TUDCA), a compound with chemical chaperone properties, reverses the ER stress-induced attenuation in leptin receptor signaling and suppresses food intake [19,21].

Markers of ER stress such as IRE1 and PERK phosphorylation, *XBP1* splicing, and the expression of CHOP and GRP78 increase in the hypothalamus of HFD-induced obese rodents [19,21,24]. It appears that dietary composition plays a fundamental role in the induction of hypothalamic ER stress. For example, saturated fatty acids such as palmitate activate ER stress in hypothalamic neurons and attenuate leptin and insulin receptor signaling [25–27]. Toll-like receptor 4 (TLR4) acts as a receptor for saturated fatty acids, and central infusion of palmitate induces inflammatory cytokine expression and ER stress through activation of TLR4 [28,29], whereas central inhibition or genetic inactivation of TLR4 confers protection against diet-induced obesity in rodents [28] (Figure 2). Likewise, neuronal (but not global) ablation of the TLR4 adapter protein MyD88 prevents excess weight gain in mice on a HFD [29,30].

Another metabolite that couples HFD consumption to ER stress is ceramide, a key intermediate of sphingolipid metabolism whose hypothalamic level is elevated in obesity [31]. Central ceramide action induces hypothalamic ER stress and causes weight gain [32]. These responses can be reversed by ventromedial hypothalamus (VMH)-specific overexpression of the predominant ER chaperone protein, GRP78 (glucose-regulated protein, also called BiP), and its dominant negative mutant induces ER stress and weight gain [32]. Overexpression of GRP78 in the VMH of leptin receptor mutant Zucker rats decreases ER stress, suppresses weight gain, and increases BAT-mediated thermogenesis without altering food intake [32]. The effects of GRP78 and ER stress in the VMH are probably independent of leptin signaling, and involve sympathetic output to adipose tissue leading to increased thermogenesis [33]. These results also suggest that improving hypothalamic ER folding capacity, for example by means of elevated GRP78 expression, is protective against diet-induced weight gain.

Obesity does not manifest central ER stress uniformly across all hypothalamic nuclei. For example, diet-induced obese (DIO) rats also display increased hypothalamic PERK and eIF2α phosphorylation [19], but these responses seem to be ARC-specific because the animals do not display ER stress in the PVN [19]. Accordingly, PVN retains its leptin sensitivity in DIO rodents [34]. Although induction of ER stress in lean animals induces markers of leptin resistance, including SOCS3 and PTP1B, and suppresses energy expenditure [19,21], central infusion of TUDCA in obese rats does not alter STAT3 phosphorylation, but acutely suppresses food intake and increases oxygen consumption [19]. The results from rodent studies using pharmacological and genetic tools collectively suggest that perturbations in ER homeostasis impair the anorexigenic response to leptin and favor positive energy balance.

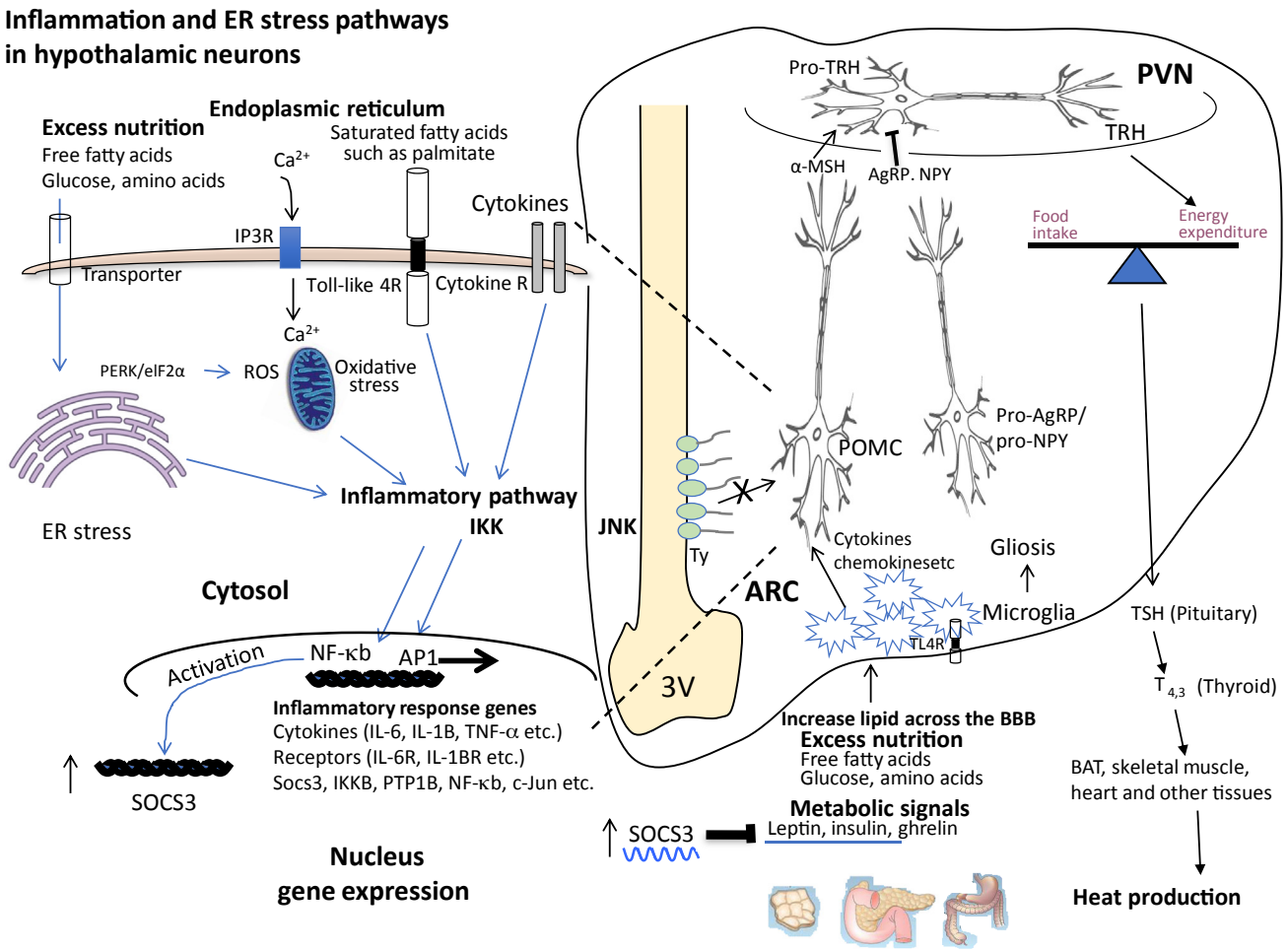

Figure 2. Hypothalamic Inflammation and Endoplasmic Reticulum (ER) Stress in Obesity. This figure depicts the hypothalamic inflammation observed in obesity that is temporally different from peripheral inflammation. Whereas peripheral inflammation is thought to develop secondary to diet-induced adiposity, rapid changes in hypothalamic inflammatory signaling cascades could be observed within 1 day of high-fat diet (HFD) consumption. For example, rodents display elevated markers of inflammation [40] and insulin resistance in the hypothalamic neuronal circuitry [71] within 24 h of HFD exposure, much earlier than the accumulation of adiposity. The central inflammation observed upon HFD consumption, at least in part, depends on the direct effect of saturated fatty acids on the hypothalamus rather than on the total calories consumed, and central infusion of saturated fatty acids to lean rodents mimics the hypothalamic insulin resistance and IKKβ activation observed in animals exposed to HFD [72]. Hypothalamic inflammation may be stimulated by acute administration of saturated fatty acids, without induction of systemic inflammation. In parallel with their central effects *in vivo*, saturated fatty acids induce ER stress in neuronal cultures [25,73]. However, their proinflammatory effect is attenuated in cultured hypothalamic neurons, suggesting a role for non-neuronal cells in fatty acid-induced central inflammation [73] (Box 2). Consumption of a high-calorie diet results in activation of microglia in the ARC [74], and diet-induced hypothalamic gliosis (activation of the glial population) is also reported in humans [40]. The resident astrocytes and microglia are the innate immune cells of the CNS, and there is a dynamic interaction between them, including during central inflammation. Activated microglia can induce a subpopulation of astrocytes by secreting inflammatory mediators, including IL-1α, TNF, and C1q, which in turn blunt for example the neuroprotective capacity of the astrocytes, leading to neuronal death [75]. In contrast to the periphery, the central innate immune response in general cannot initiate adaptive immunity, consistent with the anti-inflammatory environment of the brain parenchyma as well as the physical limitations imposed by the BBB that block migration/communication of innate and adaptive immune cell components. Genetic and pharmacological suppression of neuroinflammation leads to metabolic improvements. These include potent weight loss and/or protection from diet-induced obesity by central or peripheral administration of a JNK2/3-specific inhibitor [76], targeted activation of the anti-inflammatory glucocorticoid receptor in GLP-1-positive cells [77], and neuron-, glia-, or hypothalamus-specific deletion of IKKβ [21,78]. The ER establishes physical contacts with other organelles at membrane contact sites (not shown in the figure). Mitofusins (MFNs), for example, tether ER and mitochondria, and participate in central metabolic regulation (discussed in the main text). The figure also depicts the hypothalamus–pituitary–thyroid (HPT) axis as an example of a central pathway regulating energy balance. The HPT axis is a strong determinant of energy expenditure through the action of thyroid hormones in tissues such as skeletal muscle or BAT: α-MSH activates whereas AgRP and NPY block TRH expression in the PVN. TRH stimulates the pituitary gland to secrete TSH, which in turn leads to triiodothyronine (T3) and thyroxine (T4) production by the thyroid gland. Abbreviations: ARC, arcuate nucleus; BAT, brown adipose tissue; BBB, blood–brain barrier; PVN, paraventricular nucleus; TRH, thyrotropin releasing hormone; TSH, thyrotropin stimulating hormone; Ty, tanycytes; 3V, third ventricle.

ER Stress and Inflammation

Inflammation in the CNS correlates positively with excess calorie intake and adiposity. A significant number of studies now suggest that obesity and the associated inflammatory state form a positive feedback loop where induction of inflammation exacerbates various abnormalities observed in obesity. ER stress and inflammation are also functionally coupled, particularly with regard to their role in metabolic disorders. For example, ER stress-induced inflammation in the liver couples obesity to insulin resistance [20], and a similar link exists in the hypothalamic circuitries regulating energy metabolism. Induction of central ER stress in lean mice by tunicamycin leads to the activation of canonical IKKβ–NF-κB signaling, a master regulator of proinflammatory gene expression, whereas relieving ER stress with the chemical chaperone TUDCA alleviates HFD-induced central NF-κB activation [21]. Overexpression of constitutively active IKKβ in the MBH induces ER stress, and neuronal deletion of IKKβ relieves ER stress [21]. Activated IKKβ–NF-κB signaling increases the expression of SOCS3, which acts to attenuate insulin and leptin signaling (Figure 2). In summary, hypothalamic IKKβ and ER stress can activate each other, forming a positive feedback loop leading to dysregulation of central energy balance, and the hypothalamic IKKβ–NF-κB axis couples ER stress to central inflammation (Figure 2).

Although the role of the UPR is understudied in non-neuronal CNS populations (Box 2), results from peripheral mediators of the adaptive immune system suggest that ER stress and the inflammatory process may extend beyond the neuronal circuitry. TLR4 signaling couples saturated fatty acids to the activation of UPR such that TLR4 activation induces the IRE1–XBP1 axis in macrophages while suppressing the ATF4 pathway [35]. Distinct from the ER-

Box 2. Glial Cells

The majority of the cells in the CNS are non-neuronal cells, called glia, that are composed of three major cell populations: astrocytes, microglia, and oligodendrocytes (ODs). Ependymal cells, typically lining the ventricles, as well as Schwann and satellite cells are also part of the glial population. The primary function of these cells is to maintain homeostasis in the nervous system. Astrocytes send projections (called astrocytic end-feet) to meet the blood capillaries, and have traditionally been viewed as a gate between the circulation and the remainder of the CNS. They express leptin, insulin, and ghrelin receptors [95–97], participate in neuronal nutrient utilization [98–100], regulate systemic glucose homeostasis [96], and play an indispensable role in the central regulation of energy homeostasis [95,101]. For example, by regulating extracellular adenosine concentration, astrocytes regulate AgRP neuronal activity and actively participate in the regulation of feeding [102].

Microglia are resident macrophages of the CNS derived from erythromyeloid precursors in the yolk sac [103]. Their function includes the maintenance as well as the plasticity of synaptic circuitry, synaptic transmission, neuronal surveillance, and neurogenesis [104–107]. Rodents are estimated to have between three and four million microglia. In contrast to differentiated adult neurons, microglia can proliferate. Microglial development and survival depend on a receptor tyrosine kinase called colony-stimulating factor 1 receptor (CSF1R) [108,109] whose inhibition leads to complete microglial depletion in adult mice. Microglial depletion in DIO mice by pharmacological CSF1R antagonism can reverse obesity [74,78].

The third glial population comprises the ODs. These cells function primarily to provide electrical insulation (myelination) to neurons. In addition to this fundamental role, ODs produce a variety of neurotrophic and growth factors such as BDNF and IGF-1 that promote neuronal survival [110–112]. NG2 glia are another glial population, also called polydendrocytes or oligodendrocyte precursors [113]. These cells are characterized by their expression of a single membrane-spanning chondroitin sulfate proteoglycan, NG2, and can differentiate into myelinating ODs and engage in tissue repair [114]. Accordingly, NG2 glia become hypertrophic following demyelinating insults [115–117]. In the hypothalamus, particularly in the circumventricular organ (the median eminence, ME), NG2 glia play a crucial role in sensing circulating leptin [118]. Dendritic projections of leptin receptor-positive neurons in the ME are in close proximity to NG2 glia. Following depletion of NG2 glia, the ARC neurons lose their response to leptin, which in turn triggers obesity [118]. This finding has immediate consequences for humans because NG2 depletion upon cranial X-ray irradiation was also proposed to contribute to weight gain in humans [118].

related XBP1 targets, TLR4-mediated XBP1 activation triggers the production of proinflammatory cytokines [36]. Furthermore, deletion of IRE1α from cells of the myeloid lineage, including macrophages but not lymphocytes (T and B cells), confers almost complete protection from HFD-induced obesity and other associated metabolic abnormalities [37]. In rodent and human CNS, XBP1 is expressed in the microglia [38,39], and whether a similar link exists between fatty acids and IRE1–XBP1 pathway in CNS immune cells, such as in microglia, is worth investigating. Although our knowledge on the role of hypothalamic ER stress in human obesity is very limited, the interaction between central inflammation and energy balance displays similarities in humans and rodents [39,40] (Figure 2). These findings collectively indicate that methods to suppress central inflammation and presumably ER stress would augment energy as well as glucose metabolism.

ER Membrane Contact Sites Affect Energy Balance

There are physical interactions, called membrane contact sites, between the ER and other organelles [41]. These direct contacts are essential for several biological processes such as lipid synthesis, and they mediate ion and metabolite transport across membranes, thereby contributing to organelle function and dynamics [41,42]. Mitofusins 1 and 2 (MFN1/2) are proteins located on the ER membrane and on the outer surface of the mitochondria which help to regulate the tethering of the two organelles. These proteins appear to play a significant role in the central regulation of energy balance in a cell type-dependent manner. AgRP-specific MFN1 or MFN2 KO mice are protected from HFD-induced obesity [43]. However, ablation of MFN2, but not MFN1, from POMC neurons results in elevated ER stress and promotes both food intake and weight gain [44]. Accordingly, overexpression of MFN2 in the ARC decreases markers of ER stress and protects against HFD-induced weight gain [44]. POMC-specific MFN2 KO mice display decreased expression of POMC mRNA but accumulation of POMC protein. Although the expression of the POMC processing enzymes PC2, PAM, and CPE is also elevated in the KO mice, α-MSH levels are significantly lower, and this accounts for their obese phenotype [44]. However, upon reversal of ER stress by chronic central infusion of chemical chaperones 4-phenylbutyric acid (4-PBA) or TUDCA, elevated α-MSH levels lead to suppression of food intake and weight loss [44]. As we discuss further in the next section, these results point to the important role of defective POMC processing in the interface between ER stress and obesity.

Hypothalamic ER Stress and Neuropeptides

ER stress-induced abnormalities in energy and glucose metabolism are causally related to altered hypothalamic POMC processing. As other prohormone precursors, POMC undergoes extensive cleavage and processing throughout the secretory pathway [45]. In HFD-induced obese rodents there is a gradual decrease in the amount of POMC processing, evidenced by accumulation of intact POMC in the MBH, accompanied by decreased amounts of its processing products ACTH and α-MSH [19,44,46] (Figure 3). Defective α-MSH production significantly contributes to the development of obesity [47], and central α-MSH replenishment can reverse the excess adiposity and impaired glucose metabolism [48]. Furthermore, although leptin can stimulate, albeit at a compromised level, the expression of *POMC* mRNA and hypothalamic ACTH levels, α-MSH levels were completely insensitive to leptin administration in obese rodents [19]. These findings suggest that there is a defect in the step leading from the POMC precursor to the production of α-MSH. Although there is no significant difference in the mRNA levels of POMC processing enzymes between lean and DIO animals, there is a significant reduction in the protein levels of PC2 in ARC of DIO animals. This defect is one of the main mechanisms linking ER stress to decreased α-MSH levels and thus to obesity. PC2 is crucial for the processing of ACTH to α-MSH [45]. Central infusion of ER stress-inducing

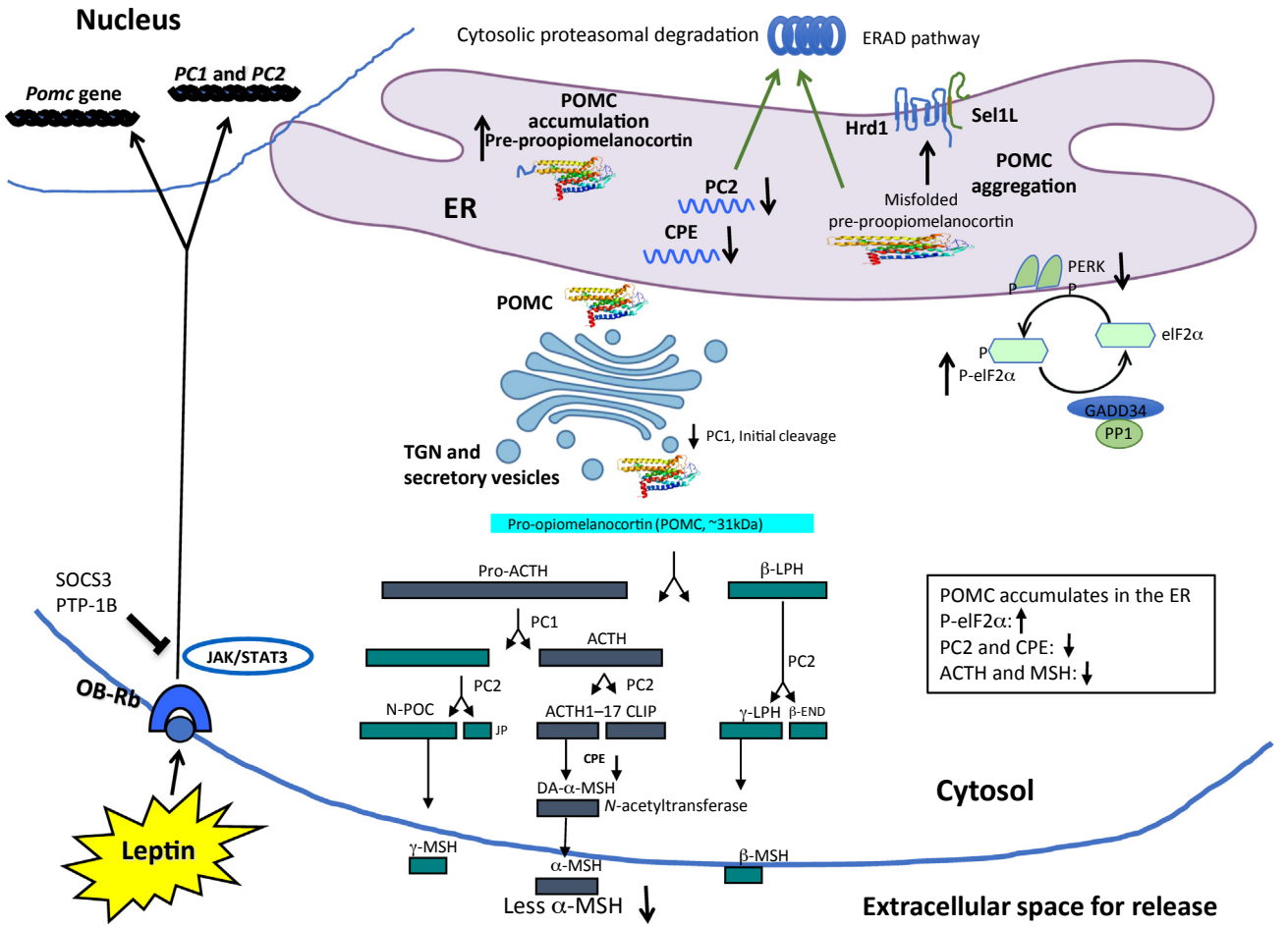

Figure 3. Endoplasmic Reticulum (ER) Stress in Proopiomelanocortin (POMC) Neurons in Response to a High-Fat Diet (HFD). This figure depicts post-translational processing of POMC in hypothalamic neurons that involves the coordinated action of several enzymes (PC1, PC2, CPE, N-acetyltransferase, and peptidyl α-amidating monooxygenase) in the *trans*-Golgi network and secretory granules [79]. The biosynthesis of POMC and prohormone convertases (PC1 and PC2) is regulated by leptin receptor signaling at the transcriptional level [80,81], and further involves ER-mediated protein folding and processing. In diet-induced obesity, a systemic rise in leptin levels is typically associated with central resistance to its anorectic action. Obesity also leads to hypothalamic ER stress, which impairs the POMC-mediated energy homeostasis through at least two distinct pathways. First, ER stress induces the expression of negative regulators of leptin receptor signaling, (e.g., through SOCS3 and PTP1B). Second, independently of transcriptional regulation, ER stress leads to decreased PC2 and CPE protein levels, the accumulation of unprocessed POMC polypeptide, and decreased POMC processing. In turn, hypothalamic α-MSH levels are dramatically decreased in obese animals. Hrd1–Sel1L are components of ER-associated degradation (ERAD), which regulates in POMC degradation. Defects in Hrd1–Se1L-mediated POMC degradation lead to POMC processing defects and accumulation of the precursor in the ER. Abbreviations: β-END, β-endorphin; P, phosphorylation.

agents (thapsigargin or tunicamycin) to lean rodents decreases PC2 and α-MSH levels to those detected in DIO animals, suggesting that ER stress negatively regulates PC2 protein levels [19] and in turn leads to decreased α-MSH levels (Figure 3). Importantly, these defects in POMC processing could be rescued by the chemical chaperones PBA or TUDCA [19]. However, the same treatment does not alter the level of hypothalamic NPY, suggesting that DIO-induced ER stress affects the processing of hypothalamic neuropeptides differently [19]. Salubrinal, an agent that selectively inhibits the dephosphorylation of p-eIF2α [49], enhances free fatty acid (FFA)-induced activation of the PERK pathway, but not the ATF6 and IRE1 branches [50]. Blocking dephosphorylation of eIF2α with salubrinal increases PC2 protein levels, and reverses

the negative effect of thapsigargin on PC2 expression, suggesting that the PERK–eIF2α branch of the UPR could protect against the decrease in PC2 during ER stress.

A recent study has further revealed the overall importance of ER homeostasis in POMC processing with regard to energy balance. Proper trafficking and processing of POMC requires a protein complex between suppressor-enhancer of Lin-like 1 (Sel1L) and hydroxymethylglutaryl (HMG)-CoA reductase degradation protein (Hrd1, also called synoviolin). Sel1L–Hrd1 is an evolutionarily conserved arm of ERAD, and their genetic deletion results in embryonic lethality in mice [51,52]. Both genes are expressed in the hypothalamus and in POMC neurons [53]. Feeding and leptin positively regulate their expression. Deletion of Sel1L from POMC neurons results in loss of response to the anorectic effect of leptin, hyperphagia, and age-associated obesity, but not in ER stress or inflammation [53]. Although *POMC* mRNA levels are unaltered in POMC-specific Sel1L deficiency, there is a significant accumulation of POMC protein. Hrd1 is an E3 ligase, and together with Sel1L targets POMC for proteasomal degradation. Consequently, in the absence of this quality control system, the nascent POMC polypeptide cannot be properly targeted for further processing, resulting in aggregation and accumulation in the ER [53]. It is possible that diet-induced obesity also results in functional dysregulation of the ERAD system in POMC neurons, contributing to the accumulation of POMC observed in obese rodents [19]. Considering the importance of POMC and its processing products in the regulation of energy balance, these results collectively suggest that proper maintenance of ER homeostasis is key to the production of active POMC-derived peptides, and ER stress and central obesity are coupled at the level of dysfunctional POMC processing.

Concluding Remarks

Diverse studies conducted in rodents as well as results obtained from humans suggest that obesity is accompanied by elevated ER stress and inflammation in the CNS. Most metabolic studies have so far focused on neuronal ER stress, and our knowledge on central ER stress in regard to the possible role of UPR in non-neuronal cells (Box 2) is relatively limited. Central inflammation encompasses neurons as well as glia, and astrogliosis and microgliosis have detrimental consequences for the hypothalamic circuits regulating energy balance. What triggers hypothalamic ER stress or inflammation in the first place is not fully understood; however, nutrient-related inputs such as saturated fatty acids appear to play a causative role. In this regard, recent findings from rodents suggesting that increased ingestion of fat (but not of sucrose or protein) leads to adiposity are worth noting [54], although whether this finding can be extended to humans is not clear. Induction of hypothalamic ER stress contributes to a positive energy balance, and this is mediated at least in part by a direct impact on altered prohormone processing, as in the case of POMC. Inflammatory mediators are classically accompanied by an anorexic response; however, prolonged activation of central inflammatory pathways results in elevated food intake and/or increased weight gain. This is a major distinction in the effect of central inflammation when considered in acute versus chronic settings. Genetic and pharmacological evidence suggests that alleviation of the hypothalamic ER stress and inflammation that accompany obesity results in weight loss, as well as in improved glucose metabolism and cardiovascular function. Results indicating that suppression of neuronal inflammation confers longevity in mice [55] highlight the importance of CNS inflammation to overall mammalian physiology, and underline the similarity between obesity and the aging process in regard to their association to central inflammation and ER stress. However, many questions remain to be answered (see Outstanding Questions) in understanding the role of ER stress and neuropeptides in the overall regulation of energy homeostasis.

Outstanding Questions

What is the primary mechanism that triggers hypothalamic ER stress and/or inflammation in response to a chronic HFD regime?

Is the decrease in α-MSH peptide levels seen in HFD a result of a POMC processing defect affecting the pro-converting enzymes, a lack of proper POMC folding and retention, or shuttling of POMC to the ERAD system for degradation?

What is the effect of ER stress on the biosynthesis of neuropeptides other than POMC? ER stress leads to a global shutdown of protein synthesis while the translation of selective transcripts is upregulated. How does this differential regulation apply to orexigenic versus anorexigenic neuropeptides?

Microglia are the immune components of the CNS; they can be depleted and renewed by pharmacological intervention, and this alters energy homeostasis. How does microglial depletion/renewal affect hypothalamic ER stress in obesity?

The extracellular domain of receptors for neuropeptides and some other metabolic hormones are subject to functional post-translational modifications, for example glycosylation, through their processing in the secretory pathway. What is the potential contribution of ER stress to the post-translational modifications of receptors regulating energy metabolism? Could such an interaction reflect a mechanistic link between ER stress and energy balance?

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