The Winter meeting of the Nutrition Society was held at the Royal College of Physicians, London on 6–7 December 2011

70th Anniversary Conference on 'Body weight regulation – food, gut and brain signalling'
Symposium III: Brain signalling and onward communications

Hypothalamic dysfunction in obesity

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A growing number of studies have shown that a diet high in long chain SFA and/or obesity cause profound changes to the energy balance centres of the hypothalamus which results in the loss of central leptin and insulin sensitivity. Insensitivity to these important anorexigenic messengers of nutritional status perpetuates the development of both obesity and peripheral insulin insensitivity. A high-fat diet induces changes in the hypothalamus that include an increase in markers of oxidative stress, inflammation, endoplasmic reticulum (ER) stress, autophagy defect and changes in the rate of apoptosis and neuronal regeneration. In addition, a number of mechanisms have recently come to light that are important in the hypothalamic control of energy balance, which could play a role in perpetuating the effect of a high-fat diet on hypothalamic dysfunction. These include: reactive oxygen species as an important second messenger, lipid metabolism, autophagy and neuronal and synaptic plasticity. The importance of nutritional activation of the Toll-like receptor 4 and the inhibitor of NF-κB kinase subunit β/NK-κB and c-Jun amino-terminal kinase 1 inflammatory pathways in linking a high-fat diet to obesity and insulin insensitivity via the hypothalamus is now widely recognised. All of the hypothalamic changes induced by a high-fat diet appear to be causally linked and inhibitors of inflammation, ER stress and autophagy defect can prevent or reverse the development of obesity pointing to potential drug targets in the prevention of obesity and metabolic dysfunction.

Hypothalamus: Obesity: High-fat diet: Inflammation: Neuronal and synaptic plasticity

The developed world is currently facing an epidemic of obesity. Diseases associated with obesity, particularly type 2 diabetes, CVD, cancer, stroke and mental health issues, including dementia[1,2] are provoking a crisis in health care, adversely affecting health and life expectancy and increasing health care costs, estimated to be up to £45 billion by 2050 in the UK alone[3]. Direct and indirect costs of type 2 diabetes, for example, one of the major health consequences of obesity, are currently £21·8 billion and set to rise to £35·6 billion by 2035–36 in the UK[4].

Obesity is the result of a disproportionately high energy intake compared to energy expenditure and is a complex interaction between environmental and genetic factors[5] with monogenic defects being relatively rare[6]. It seems obvious that an energy-dense diet, high in saturated fat and sugar, should cause weight gain and increased adiposity; nonetheless it appears that these diets cause a profound change in energy balance that does not result from a simple increase in energetic intake. Diet composition, particularly the presence of long-chain saturated fats, results in metabolic dysfunction and increased adiposity and body weight,

which is defended so that any subsequent weight loss through energetic restriction is difficult to maintain[7].

It is well known that the hypothalamus regulates energy balance, carrying out this process by integrating peripheral hormonal and neuronal signals of satiety and nutritional status[8–10], and by directly sensing nutrients[11,12]. The hypothalamus not only regulates food intake and energy expenditure but also the utilisation and partitioning of nutrients[13], the regulation of glucose homoeostasis[14] and peripheral lipid metabolism[15–17]. Two important anorexigenic hormones that signal adiposity and nutritional status to the hypothalamus and inhibit food intake are leptin, produced by white adipose tissue (WAT), and insulin, produced by the pancreatic β-cells. While circulating levels of leptin and insulin rise in obesity, insensitivity to both hormones rapidly develops making it a hallmark feature of obesity. In addition to the classical target tissues for insulin action, such as the liver, muscle and WAT, insulin also acts in the hypothalamus playing a pivotal role not only in maintaining energy balance but also in regulating peripheral lipid and glucose metabolism[18–20]. Leptin is not only a potent regulator of food intake and possibly energy expenditure[21], but also acts in concert with the central action of insulin in the maintenance of peripheral glucose homoeostasis[14,22]. Ghrelin is the only peripheral orexigenic hormone. It is produced mainly in the stomach and acts in the hypothalamus to stimulate food intake and weight gain via increased adiposity[23]. It is released from the stomach immediately preceding meals in human subjects[24] and is n-octanoylated to produce the form of ghrelin which is active in energy balance[23]. Ghrelin acts via its receptor GHS-R in the hypothalamus and, counter intuitively, insensitivity to ghrelin also develops in diet-induced obesity[25].

The complexity of, and numerous levels of control over, energy balance in the hypothalamus begs the question as to how this system can be so easily compromised and degraded in obesity. A key factor in the obesity epidemic, and the cause of the hypothalamic failure to regulate energy balance, appears to be the availability of highly palatable, energy-dense foods high in saturated fats and refined sugars. In the USA, an increase in total energy intake has occurred, largely since the 1980s, which tracks with the increase in obesity indicating that food is a pivotal factor in the obesogenic environment[26]. Recently 'over nutrition' has been recognised as not just a contributory factor to obesity but also as a mechanistic link stimulating the innate immune system and causing an atypical inflammation that is fundamental in the development of obesity and metabolic disease. In human subjects, chronic systemic low grade inflammation is marked by an increase in inflammatory markers in the circulation, such as C-reactive protein, TNFα, IL-1 and IL-6 in obese individuals, although a considerable overlap in values can occur between lean and obese. Nevertheless, a correlation exists between high levels of inflammatory markers and increasing BMI[27]. Thus, it is now widely accepted that obesity is related to low levels of systemic inflammation[28,29], and a new concept of immunometabolism has emerged recognising the close functional links between the two processes[30]. The most widely studied dietary components in the induction of inflammation are the long-chain SFA[31].

It is only relatively recently that inflammation has also been shown to occur in the hypothalamus in obesity[32–34]. Indeed hypothalamic 'injury' has been identified in rodents on a high-fat diet as early as 1 d after the start of feeding[35]. The inflammation in the hypothalamus caused by diet differs from inflammation due to sickness[36]. In the sickness response, food intake is inhibited and body weight is lost mainly due to the energetic demands of sustaining an elevated temperature, whereas diet-induced hypothalamic inflammation results in obesity[37]. Thus, over nutrition must be able to cause inflammation via subtly different mechanisms compared to the sickness response. The role of over nutrition and its impact on the hypothalamus has taken centre stage as the causative mechanism in the development of obesity and metabolic dysfunction[38]. Part of this recognition has come from the identification of a number of processes elicited in the hypothalamus by diet, particularly the pro-inflammatory effects of a long-chain SFA and the role of lipid metabolism in the hypothalamus and its importance as a basic cellular mediator of energy regulation. Additionally the role of reactive oxygen species (ROS) in hypothalamic cellular signalling in energy balance[39,40] and the effect of oxidative stress when ROS levels rise uncontrollably has been identified. Endoplasmic reticulum (ER) stress has also been shown to occur in the hypothalamus as well as in peripheral tissues in response to over nutrition[41]. Finally, the recognition that both neuronal and synaptic plasticity are integral to hypothalamic energy balance and that these processes involve the resident inflammatory and support cells of the brain, the microglia and the astrocytes, respectively, and that both cell types are involved in the inflammatory response to a high-fat diet[42–45], has brought all of these processes together. In combination, they demonstrate that over nutrition can damage the neuronal substrate of energy balance at many levels ranging from the intracellular to the structure of key neuronal interactions, thus, contributing to obesity and metabolic dysfunction. Importantly these mechanisms are only now beginning to be characterised, and in doing so many potential drug targets may be revealed to combat obesity and related diseases. This review will bring together literature supporting the role of the hypothalamus as the key area for nutrient interaction in obesity and metabolic dysfunction. Stressors linking diet to hypothalamic dysfunction are also discussed. The object of the present review is to provide a summation of the processes involved in the interaction between a diet high in SFA and hypothalamic dysfunction. Many of the mechanisms described are complex and are in themselves the subject of recent review. Consequently, a detailed description of each separate process is beyond the scope of this review which aims to provide an overall picture of how diet can damage the hypothalamus.

Obesity, inflammation and insulin insensitivity

Cellular mechanisms

The fundamental links between obesity, inflammation and insulin insensitivity have been the subject of numerous research papers and reviews. Two main pro-inflammatory intracellular signalling mechanisms, the c-Jun

amino-terminal kinase (JNK) 1 and inhibitor of NF-κB kinase subunit β (IKKβ)/NF-κB pathways, have been identified as being key in linking diet to metabolic dysfunction[46]. Both pathways are activated by metabolic stress and are causal in the induction of diet-induced obesity.

The JNK group of stress-activated kinases are part of the mitogen-activated protein kinase family and are stimulated by growth factors, pro-inflammatory cytokines, particularly TNFα, microbial factors, including lipopolysacccharide (LPS) and other stressors notably oxidative and ER stress[47]. The JNK are thought to increase inflammation by stabilising mRNA encoding pro-inflammatory cytokines and other mediators of inflammation[46–48].

IKKβ is an integral part of the regulatory complex responsible for activation of the NF-κB inflammatory pathway, an important part of the innate immune response. IKKβ phosphorylates and inactivates the inhibitor of κB which prior to phosphorylation sequesters NF-κB in the cytoplasm. When freed NF-κB dimerises and enters the nucleus where it regulates transcription of a number of genes related to inflammation[49]. IKKβ is stimulated by pro-inflammatory cytokines, viruses and bacterial components, including LPS, and stressors such as oxidative and ER stress.

It has been known for some time that inflammation caused by infection and disease results in insulin insensitivity[50] and that both diet-induced inflammation and insulin insensitivity can be reversed by anti-inflammatory agents such as salicylate[51,52], demonstrating the connection between the two. Salicylate inhibits the activity of IKKβ[53], which in addition to its role in inflammation also plays an important part in the insulin-signalling cascade[46] inhibiting insulin receptor substrate 1 in adipocytes and hepatocytes[54]. IKKβ is also part of the mammalian target of rapamycin (mTOR)-Raptor complex[55] and the mTOR pathway is part of a mechanistic link between insulin and leptin signalling[56]. mTOR also triggers ER stress and inhibits insulin signalling via JNK-mediated serine phosphorylation of insulin receptor substrate 1[57]. Stimulation of the IKKβ/NF-κB inflammatory pathway also induces SOCS3, an inhibitor of both leptin and insulin signalling[33]. JNK1 appears to act in the brain, particularly in the agouti-related peptide (AgRP) neurons to increase the inhibitory effects of insulin on food intake[32,58], but under different conditions can have opposing effects on food intake and is proposed to be both a positive and a negative regulator of food intake[58]. Nonetheless, evidence indicates that it is the production of inflammatory cytokines via the IKKβ/NF-κB and JNK pathways that is the major link between inflammation and insulin insensitivity[46,59].

White adipose tissue as a source of inflammation

Inflammation is normally a transient response to infection or injury and is rapidly resolved to prevent tissue damage and chronic disease[60]. However, in obesity although the inflammation is low grade it persists. Adipocytes are cells which not only store fat but also secrete a number of pro-inflammatory cytokines and adipokines in response to over nutrition and over expansion. This attracts monocytes into the tissue to become M1, pro-inflammatory, macrophages which form typical 'crown-like' structures surrounding necrotic and apoptotic adipocytes, releasing further pro-inflammatory cytokines[61,62]. The causes underlying the secretion of pro-inflammatory adipokines by WAT may be due to WAT over expansion causing hypoxia[63] and/or high concentrations of NEFA being released through uncontrolled lipolysis due to insulin insensitivity in adipocytes[64]. However, dietary restriction in obese rodents has also been found to cause a rapid but transient influx of macrophages into WAT with a correlation between NEFA concentration and peak macrophage numbers, confirming the role of high concentrations of NEFA in this effect[65]. One of the pro-inflammatory cytokines released by WAT is TNFα. The concept that inflammation in WAT in obesity is causative in insulin insensitivity was first identified in rodent models of obesity where TNFα was elevated and both antibodies directed against TNFα and knockout of the TNFα gene prevented the induction of insulin insensitivity[66,67]. However, TNFα neutralisation in obese human subjects, while reducing the level of inflammatory markers, has no effect on insulin sensitivity[68]. Nonetheless the systemic inflammation in obesity due to the release of adipokines is still thought to be an important mechanism in metabolic dysfunction. However, in addition to the pro-inflammatory role of WAT pathways and mechanisms are still being uncovered which implicate diet directly with inflammation.

Food and inflammation

Nutrition can have an immediate effect on circulating markers of inflammation. Within hours of eating a fatty meal a transient increase in inflammatory markers is seen in the circulation. This reaction appears to be preferentially activated by TAG, SFA and glucose which trigger an acute innate response in circulating monocytes releasing TNFα and IL-6[69–71]. The most potent stimulator of the Toll-like receptor 4 (TLR4), the major signalling pathway in the innate immune response, is bacterial LPS or endotoxin. The potential for LPS, either ingested as a contaminant, particularly of highly processed food[72], or from the gut microbiota entering the circulation after a high-fat meal has been demonstrated in rodent models of obesity[73] and in human subjects[74]. Increased bodyweight, glycaemia and aging all increase the magnitude of the post-prandial inflammatory response indicating that the innate immune system is sensitised in these conditions[27]. Long-chain SFA have been shown to act as ligands for TLR4[75–79] and mice that lack a functional form of this receptor are protected from obesity[80] and from obesity-related insulin insensitivity[81], administration of LPS has been shown to mimic the effects of a high-fat diet on adiposity and insulin insensitivity[73]. There is a possibility that dietary SFA act synergistically to amplify the response to LPS[82] as transfected cells and cells naturally expressing TLR4 fail to respond to the fatty acids alone[83]. Whatever the ligands are that activate TLR4 its presence has been shown to be necessary for acute lipid-induced insulin insensitivity[78].

The TLR4 response is dependent on the IKKβ/NF-κB pathway and IKKβ activation can also disrupt insulin signalling as detailed earlier[84]. Knockout of the genes
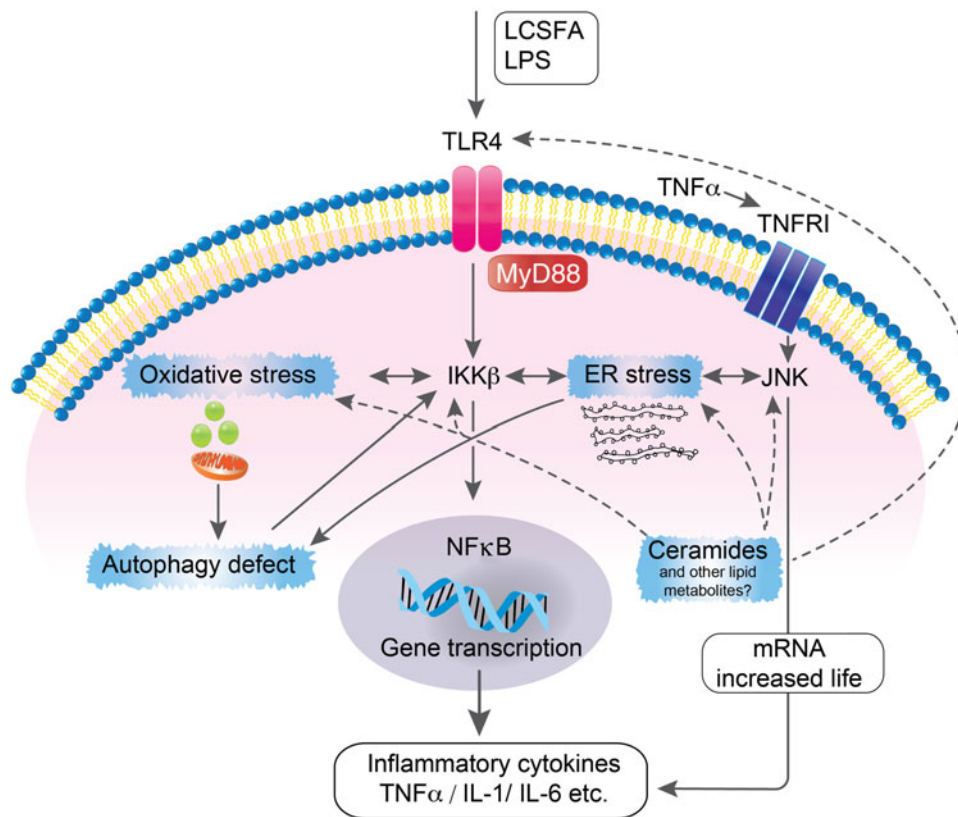
Fig. 1. (colour online) The major intracellular pathways involved in the induction of hypothalamic dysfunction are illustrated. All of these pathways interact with one another to amplify the response to a high-fat diet and obesity. Interactions between pathways are shown in both solid and dotted lines. Key: peroxisomes are shown in green and mitochondria in orange. ER, endoplasmic reticulium; IKKβ, inhibitor of nuclear factor κB kinase subunit β; JNK, c-Jun amino-terminal kinase; LCSFA, long-chain SFA; TLR4, Toll-like receptor 4; TNFR1, tumour necrosis factor receptor 1.

encoding IKKβ and TLR4 in myeloid cells, which give rise to macrophages and neutrophils, protects mice from high-fat diet-induced insulin insensitivity in the muscle, fat and liver[79,85]. By contrast, IKKβ knockout in the liver protects only the liver[85], indicating that the myeloid-derived cells of the immune system are necessary for diet-induced obesity and metabolic dysfunction. Deletion of JNK1, another key component of the inflammatory response stimulated via the TNFα receptor 1 also protects from obesity and insulin insensitivity[86]. In mice on a high-fat diet, insulin insensitivity mediated via TLR4 can be detected as early as 3 d after the start of diet, and hypothalamic inflammation after just 1 d, indicating that the diet rather than increased adiposity is the cause of insulin insensitivity and inflammation[35,87]. Both the IKKβ/NF-κB and the JNK pathways can also be stimulated via non-receptor stress mechanisms within the cell including oxidative stress, ER stress, and excess concentrations of ceramides all of which can be caused by excessive long-chain saturated fats in the diet[46,88] (Fig. 1).

Hypothalamic control of energy balance

The hypothalamus contains several nuclei which play important roles in energy balance. The most important of these are the arcuate nuclei (ARC) which consist of groups of neurons within the mediobasal hypothalamus lying just above the median eminence and either side of the third ventricle (Fig. 2). Although other nuclei in the hypothalamus are inside the blood–brain barrier, the ARC lie, at least partially, outside and are perfectly placed to receive input from circulating hormones and nutrients and also from those in the cerebrospinal fluid present in the third ventricle.

Two sets of neurons with opposing actions have been identified as being pre-eminent in energy balance regulation. One group of neurons stimulate appetite and are termed orexigenic and express neuropeptide Y (NPY) and AgRP, while the second group inhibit appetite and are termed anorexigenic and express proopiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART). Both sets of neurons possess the long signalling form of the leptin receptor, Ob-Rb, and leptin acts to inhibit the AgRP/NPY neurons while activating the POMC/CART neurons. These effects are seen at the level of both gene transcription and membrane polarisation[89]. Projections from the ARC reach the paraventricular nuclei which contain high levels of the melanocortin receptors 3 and 4. The melanocortin receptor 4 is the key receptor in energy balance with mutations of this receptor being the
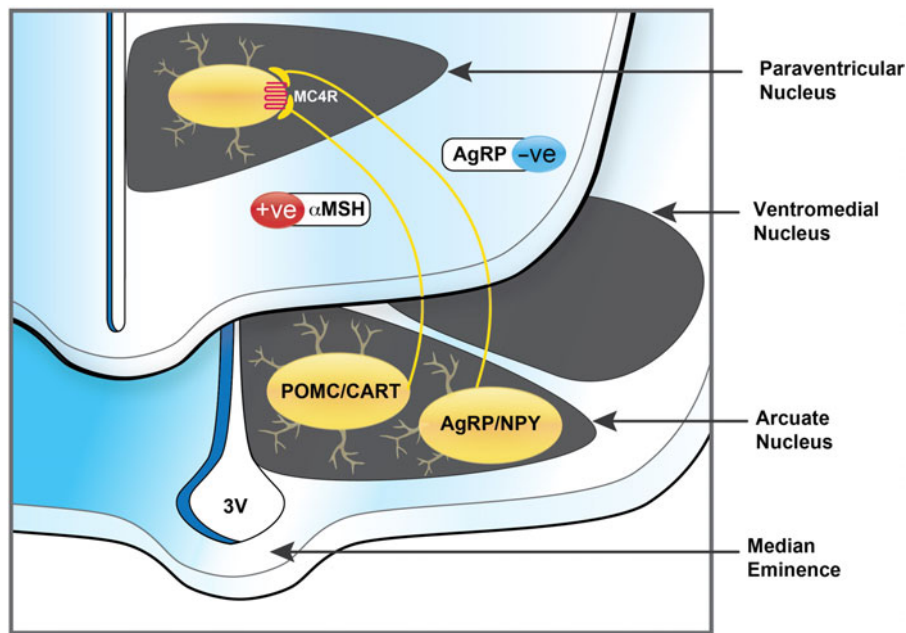
Fig. 2. (colour online) The major neuronal cell types that control energy balance are part of the melanocortin system in the hypothalamus. They are mostly located in the arcuate and ventromedial nuclei. They are referred to as first-order neurons as they receive direct input from circulating hormones such as leptin, insulin and ghrelin. They then signal second-order neurons in areas such as the paraventricular nuclei. 3 V, third ventricle; AgRP, agouti-related peptide; αMSH, α melanocyte-stimulating hormone; CART, cocaine and amphetamine-regulated transcript; MC4R, melanocortin receptor 4; NPY, neuropeptide Y; POMC, proopiomelanocortin.

most frequent seen in human obesity[90]. POMC-derived α melanocyte-stimulating hormone acts as an agonist at these receptors and AgRP as an antagonist[91], providing a dynamic balance to regulate energy balance. AgRP, the POMC gene and its product α melanocyte-stimulating hormone and the melanocortin receptors 3 and 4 are part of the central melanocortin system[91].

Synaptic plasticity

The AgRP/NPY neurons contain GABA, an inhibitory neurotransmitter[92]. POMC/CART and AgRP/NPY neurons interact directly with one another in energy balance, with the AgRP neurons maintaining a tonic inhibitory effect on the anorexigenic POMC/CART neurons, via γ-aminobutyric acid, resulting in a stimulation of feeding. It has recently been shown that POMC/CART neurons can also directly inhibit AgRP/NPY. The balance of the interaction between these two neuronal types is dependent on synaptic plasticity. Although it has been known for some time that synaptic plasticity in the hypothalamus is important in osmotic regulation, reproductive behaviour and circadian rhythmicity[93], its role in energy balance has only recently begun to be explored. Detailed analysis of the neurons in the ARC, particularly those of the melanocortin system, has been conducted in transgenic mice with NPY and POMC cells labelled with green fluorescent protein and visualised using fluorescence and electron microscopy. Both leptin and ghrelin have been shown to regulate the connectivity between AgRP/NPY and POMC/CART neurons in the ARC[42] with astrocytes playing a

pivotal role in this process by surrounding and ensheathing neurons and their processes and regulating synaptic input[93].

The complex nature of the synaptic plasticity mediating the effects of both ghrelin and leptin has been recently demonstrated. In response to fasting, and an increase in circulating levels of ghrelin, a persistent up-regulation of an, as yet unidentified, excitatory synaptic input to AgRP neurons was observed. This presynaptic pathway releases glutamate that stimulates the AMPA receptor. This induces a positive feedback loop with AMP-activated kinase, mediating Ca release which activates the AgRP neuron and stimulates feeding behaviour. This process, including increased synaptic connectivity, is long lasting and self-sustaining which means that a transient ghrelin signal can sustain a long lasting increase in feeding behaviour. An increase in circulating leptin arrests this activity by stimulating POMC neurons to release the opioid neurotransmitter, β-endorphin, which switches off the AMP-activated kinase pathway, AgRP activity and feeding behaviour[44] (Fig. 3).

Synaptic organisation of the melanocortin system in rats resistant to diet-induced obesity compared with those vulnerable to diet-induced obesity differed in both the quantitative and qualitative synapses on POMC neurons. Ensheathment of POMC neurons by glial cells plays a key role in the process. In addition, the reactive gliosis observed on a high-fat diet (detailed later) results in restricted access of both POMC and NPY neuronal bodies and dendrites to the blood vessels[94]. Astrocytes are the most numerous cells in the brain and play roles intrinsic to
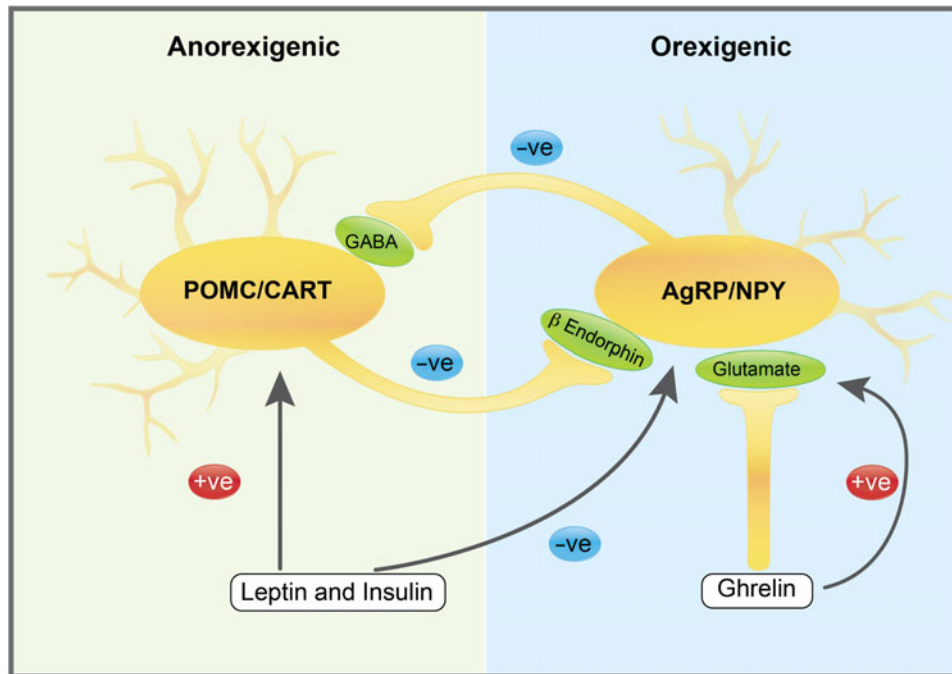
Fig. 3. (colour online) Synaptic plasticity is important in the maintenance of energy balance in addition to direct input from peripheral hormones such as leptin, insulin and ghrelin. This figure illustrates how the two major energy balance neuronal types in the hypothalamus interact with one another via synaptic inputs which are largely inhibitory. AgRP, agouti-related peptide; CART, cocaine and amphetamine-regulated transcript; GABA, γ-aminobutyric acid; NPY, neuropeptide Y; POMC, proopiomelanocortin.

its function, particularly synaptic plasticity. Microglia are the resident immune cells in the central nervous system and when activated they profoundly change their morphology, release cytokines and pro-inflammatory factors that may be neurotoxic[95]. In the hypothalamus, both astrocytes and microglia have been shown to be activated by a high-fat diet to the extent that after 1 week on the diet they form a reactive gliosis, normally seen after brain insult such as ischaemia or excitotoxicity[35]. This diet-induced gliosis resolves but returns later when obesity has more fully developed. Importantly it has been shown to occur in the hypothalamus of obese human subjects demonstrating that the changes observed in the diet-induced rodent hold true in human obesity[35]. Thus, hypothalamic inflammation can impinge on synaptic plasticity, an important mechanism in the regulation of energy balance.

Neuronal plasticity

It has been known for some time that treatment with ciliary neurotrophic factor (CNTF) can lead to weight loss[96,97] and that its potent action continues for some time after treatment has been terminated[98]. CNTF shares common signalling pathways and anatomical localisation of receptors in the hypothalamus with leptin, but unlike leptin it can act in high-fat diet-induced obese rodents, which are leptin insensitive, and in *db/db* mice which lack functional leptin receptors[99,100]. CNTF is known to support neuronal survival both *in vivo* and *in vitro*[101] and has been shown

to increase neurogenesis in the adult mouse hypothalamus which is intrinsically linked to the effect of CNTF in weight loss, as anti-mitotic agents can obliterate the effects of CNTF[97]. This strongly indicates a role for neurogenesis in the hypothalamic regulation of energy balance[97]. Up until this point very little attention had been paid to the potential role of neurogenesis in the adult hypothalamus, which had only been reported in two studies[102,103]. By using central instead of peripheral administration of bromodeoxyuridine, which is incorporated into replicating DNA, a much higher rate of neurogenesis has been found throughout the hypothalamus[104]. As neurogenesis occurs in the hypothalamus, then neuronal turnover and apoptosis must also take place to maintain the constant size of the hypothalamus. As a high-fat diet has been shown to induce inflammation in the hypothalamus and the mechanisms underlying inflammation and apoptosis are related, sharing common signalling pathways[105,106], the rate of apoptosis was studied in the hypothalamus of both rats and mice on a high-fat diet[107]. The expression of a number of key pro-apoptotic genes was increased and the TUNEL assay showed increased numbers of apoptotic cells in the ARC and in the lateral hypothalamus, the majority of which were neurons[107]. When both measures of neurogenesis and apoptosis were integrated, a surprisingly large amount of neuronal remodelling of energy balance centres was demonstrated in adult rodents[43]. A high-fat diet both suppressed adult neurogenesis by increasing apoptosis in newly divided cells and depleted the number of proliferating progenitor glial cells, resulting in mice retaining

older neurons while younger neurons underwent apoptosis[43]. The origin of this neurogenesis was identified as being the tanocytes, a specialised type of microglia, lining the base of the third ventricle in the median eminence[108].

Hypothalamic fatty acid metabolism

SFA increase inflammation and insulin insensitivity in a number of cells and tissues. The role of NEFA in the induction of insulin insensitivity in peripheral tissues is a widely accepted concept[109,110]. However, recent evidence indicates that insulin insensitivity may not be directly related to the concentration of circulating NEFA[111] and that the accumulation of diacylglycerol in peripheral tissues can induce insulin insensitivity in the absence of inflammation[112]. The ectopic accumulation of lipids in peripheral tissues and its effects on insulin sensitivity is termed lipotoxicity[113].

It had been thought that NEFA were not normally used by the brain as an energy source and did not enter the brain. Recently, however, the uptake of fatty acids by the brain has been demonstrated using stable isotopes and positron emission tomography and was found to be increased in individuals with the metabolic syndrome and to subsequently decline after weight loss[114]. Apart from the use of lipids as an energy source in the brain it has become increasingly apparent that lipids and lipid metabolism play an important role in the regulation of energy balance by the hypothalamus. Circulating TAG have been shown to inhibit food intake by regulating the level of orexigenic peptides in the hypothalamus[115] and long-chain SFA administered directly to the hypothalamus were shown to inhibit food intake[116] demonstrating the role of lipids in the hypothalamic sensing of nutrient overload.

The more extensive role of fatty acid metabolism in the hypothalamic regulation of energy balance was first indicated by the inhibition of food intake by the central administration of C75, a fatty acid synthase inhibitor[117]. All of the enzymes of the fatty acid synthesis pathway are expressed in the ventromedial nuclei of the hypothalamus. These include acetyl-CoA carboxylase, fatty acid synthase and malonyl-CoA decarboxylase[118,119]. Additionally, it has been demonstrated that nutritional status regulates hypothalamic malonyl-CoA levels and fatty acid synthase expression in a nucleus-specific manner, with fatty acid synthase and acetyl-CoA carboxylase levels down-regulated by fasting and up-regulated by refeeding[119]. The extensive role of lipid metabolism in the hypothalamus has recently been revealed in mediating responses to both fasting[120] and peripheral hormones, particularly ghrelin[119,121,122], with elevation of the long-chain fatty acyl Co-A in the ARC decreasing food intake and whole body glucose metabolism[19].

The AgRP/NPY neuron is important in stimulating feeding behaviour and is activated in response to fasting[123,124], with ghrelin stimulating feeding behaviour by stimulating AgRP/NPY neurons to suppress POMC/CART neurons[125,126]. However, in fasting, circulating levels of glucose may be low and AgRP/NPY neurons have been shown to take up fatty acids from the circulation, which are raised in fasting due to lipolysis of WAT, and convert them to TAG for storage as cellular lipid droplets which are then metabolised via autophagy[120]. This is also seen as a process by which the neurons can protect themselves from the presence of NEFA. When autophagy is blocked specifically in AgRP/NPY neurons, mice are leaner and weigh less even when food is readily available, indicating that autophagy in these neurons is necessary for AgRP to stimulate feeding[120]. When autophagy is specifically blocked in POMC neurons, leptin signalling is inhibited and mice have increased body and fat mass[127]. However, when autophagy in the mediabasal hypothalamus as a whole is blocked by short hairpin RNA, the IKKβ/NF-κB pathway is stimulated and obesity results[128]. Thus, lipid biosynthesis and utilisation via autophagy in neurons in the hypothalamus are necessary for regulation of energy balance.

Hypothalamic inflammation and insulin insensitivity have been linked to the accumulation of the saturated long-chain palmitoyl- and stearoyl-CoA[34], implicating lipids in the induction of inflammation in the hypothalamus. However, lipids are normally metabolised by the astrocytes which supply the neurons with ketone bodies particularly during suckling and starvation when lipids are the main energy supply[129]. Thus, these saturated long-chain acyl Co-A may only be present in quantity in astrocytes. Recently, it has been shown in rats that TLR4 stimulation is an upstream signalling event in SFA-induced ceramide biosynthesis in skeletal muscle, liver and hypothalamus, and that insulin insensitivity mediated via the TLR4 requires the biosynthesis of SFA-induced ceramide in liver and muscle. Nonetheless, blocking the biosynthesis of ceramide did not block the increase in SFA-stimulated circulating cytokine levels via TLR4[130]. Conversely, LPS also causes an increase in cellular ceramide levels and blocking the rise in ceramide effectively negates the influence of LPS on insulin sensitivity. It has been suggested that hypothalamic lipotoxicity could be an important link between a high-fat diet and neuronal dysfunction[131]. Hypothalamic lipidomics has the potential to identify changes in lipid species and metabolism in the hypothalamus in response to a diet high in saturated fat. Interestingly, high-fat diet-induced obesity can be reversed when saturated fat is replaced by unsaturated fat. This included an improvement of hypothalamic inflammation and the restoration of leptin and insulin sensitivity[132].

Hypothalamic autophagy

Apart from a role in lipid metabolism, detailed earlier, autophagy is considered important in the general 'housekeeping' of the cell, removing damaged organelles and cytoplasm which is particularly relevant in metabolic stress[133]. Autophagy is an important response to cellular stress, particularly ER and oxidative stress, both of which are induced by over nutrition[134,135]. However, if cellular stressors continue over long periods then what is known as the autophagy defect occurs, which effectively negates the ability of the cell to remove damage. The autophagy defect can also activate the IKKβ/NF-κB pathway[136]. Recently the importance of autophagy in the hypothalamus was revealed by specific mediobasal hypothalamic knockout of

a key gene, *Atg7*, in autophagy. Mice with this defect ate more and gained weight and showed hypothalamic activation of the IKKβ/NF-κB pathway. The effect of defective autophagy was reversed by inhibition of IKKβ[128], clearly linking defective autophagy to hypothalamic inflammation.

Hypothalamic oxidative stress

The human brain represents approximately 2 % of the body weight, but accounts for around 20 % of the oxygen and energies consumed by the body[137]. This high rate of metabolism results in a susceptibility to oxidative stress[138]. Indeed oxidative stress is a key feature of many neurodegenerative diseases[139]. Mitochondria generate super oxide anions and hydrogen peroxide (O_2 and H_2O_2), ROS, as by-products of energy generation. Usually the production and clearance of ROS, by antioxidant enzymes, is balanced between the mitochondria and peroxisomes, and plays an important role in cellular functions. Nonetheless persistent ROS can cause protein, lipid and DNA peroxidation and damage. Oxidative stress has been reported to precede the appearance of insulin insensitivity in high-fat diet-induced obesity[140]. The induction of hypertryglyceridaemia in rats has been shown to increase mitochondrial respiration in the hypothalamus together with an increase in ROS production[141]. ROS have been shown to be important in both glucose and lipid sensing by the hypothalamus[141,142] and increased ROS in the hypothalamus of the Zucker fatty rat has been linked to abnormal glucose sensing[143]. Indeed in the hypothalamus, the regulation of the melanocortin system requires the presence ROS as an acute activator of firing by POMC neurons resulting in decreased food intake, and suppression of ROS leads to activation of the AgRP/NPY neurons and increased feeding. In lean mice, hypothalamic ROS is positively correlated with leptin levels, but levels drop after a high-fat diet as PPARγ is up-regulated together with the related increase in peroxisomes which act as ROS scavengers[144]. This decrease in ROS increases food intake and may be a mechanism by which hypothalamic leptin insensitivity is induced[39]. The recent finding that certain neurons utilise intracellular lipid as a source of energy during fasting and the fact that β oxidation of fatty acids promotes the generation of ROS[145,146] demonstrates the potential for diets high in saturated fat to impact on hypothalamic fatty acid metabolism and oxidative stress and alter ROS signalling in the hypothalamus.

Hypothalamic endoplasmic reticulum stress

The cellular ER produces proteins for secretion and for intracellular function, and when stressed initiates a complex adaptive signalling response also known as the unfolded protein response (UPR). This process enables the cell to regulate the abundance of ER to fulfil its requirements for protein synthesis[147]. When the UPR continues uncontrolled it can lead to apoptosis and cell death as in the case of type 2 diabetes where increasing demands are placed on the pancreatic β-cells to produce insulin and the UPR eventually leads to pancreatic β-cell death[148]. The UPR in the liver has been shown to interfere with both insulin signalling and to promote weight gain[29] with ER stress seen as a critical intracellular event that appears to link over nutrition to metabolic dysfunction[149,150]. The UPR regulates the expression of genes that encode chaperones, which help proteins to fold correctly but also stimulates the IKKβ/NF-κB and JNK inflammatory pathways. The UPR can be stimulated by a number of metabolic stressors including high levels of protein synthesis, high concentrations of glucose, starvation, hypoxia and elevated intracellular lipids[151]. In the hypothalamus, the UPR results from a high-fat diet and has been shown to be both a cause and a consequence of the NF-κB inflammatory pathway[33]. ER stress is also causative in the inhibition of leptin and insulin signalling via induction of PTP1B[152]. Central administration of drugs that induce ER stress recapitulate the effects of a high-fat diet on the IKKβ/NF-κB pathway[33] and ER chaperones which reverse the UPR when delivered centrally reduce food intake and weight gain[33].

Hypothalamic inflammation

Hypothalamic dysfunction is pivotal in the development of obesity and peripheral insulin insensitivity. Sensitivity to the circulating anorexigenic hormones, leptin and insulin, and the orexigenic hormone, ghrelin, is lost early in diet-induced obesity and high-fat feeding[25,153,154]. Insulin and leptin signalling in the hypothalamus are integrated via the phosphatidylinositol 3-OH kinase[155,156], forkhead box protein O1[157,158] and the mTOR pathways[56]. There also appear to be at least two inhibitors which target both insulin and leptin signalling; SOCS3[159,160] and PTP1B[161] with all of these pathways implicated in the induction of diet-induced leptin and insulin insensitivity.

While the role of obesity-related inflammation has been recognised in many peripheral tissues particularly WAT and liver, the role of inflammation in the hypothalamus has taken longer time to develop. Lack of response to the anorexic effects of insulin in the hypothalamus together with up regulation of JNK by a Western diet was noted in rats after just 10 d[162] and has been recently shown to occur within 1–3 d of high-fat diet, but temporarily diminished to return permanently as obesity progressed[35]. Activation of inflammatory pathways in the hypothalamus of rats after 16 weeks on a high-fat diet has also been identified using macroarrays with an up-regulation of the inflammatory cytokines TNFα, IL-1β and IL-6, and inflammatory response proteins. These changes were correlated with the inability of an insulin challenge to inhibit food intake or to invoke a number of key steps in the insulin signalling pathway in the hypothalamus[32]. Blocking the inflammatory pathway in the hypothalamus with a specific inhibitor of JNK lowered adipose weight gain on the high-fat diet and preserved insulin signalling pathways. Pair-feeding experiments demonstrated that the effects of the JNK inhibitor were not due to the reduction in food intake. Leptin insensitivity in these animals, however, could not be reversed by the JNK inhibitor[32]. When central inflammation was induced by administration of TNFα, a known activator of JNK, it surprisingly decreased food

intake and increased energy expenditure, but when given at lower doses it partially but significantly inhibited the anorexigenic actions of both leptin and insulin[163]. Inflammation, via TNFα, has also been shown to raise the levels of hypothalamic PTP1B, a major negative regulator of insulin and leptin sensitivity[164–166].

Maintaining sensitivity to leptin depends on the prevention of diet induced ER stress[167] and IKKβ activity[33]. Neuron-specific knockout of myD88, an adaptor protein that couples stimulation of the TLR and the IL-1 receptor to downstream intracellular events, protects mice from central obesity and both leptin and insulin insensitivity[168]. The hypothalamus expresses IKKβ at relatively high levels and when exposed to dietary or genetic obesity or nutrient stimulation (e.g. glucose or lipid), IKKβ is activated[33]. Pharmacological activation of the IKKβ/NF-κB pathway or introduction of constitutively active IKKβ causes obesity and leptin and insulin insensitivity while inhibition of the NF-κB pathway maintains leptin and insulin sensitivity and protects against obesity[33]. However, stimulation of this inflammatory pathway was not found to increase expression of hypothalamic cytokines[33].

Microglia, the resident macrophages in the brain, are the major cells that express TLR4 and thus are the LPS responsive cells in the hypothalamus[169]. Confirming this, isolated neurons do not respond directly to long-chain SFA by increased inflammation or insulin insensitivity[170] indicating that an indirect mechanism involving non-neuronal cells, almost certainly the microglia, is involved. Nonetheless, prolonged exposure of neurons to SFA does cause ER stress and apoptosis although the mechanism was not identified[170,171]. In the hypothalamus, both astrocytes and microglia have been shown to be activated by a high-fat diet[35] indicating that both synaptic and neuronal plasticity, recently identified key mechanisms in energy balance, are almost certainly compromised.

Conclusions

The hypothalamic regulation of energy balance is rapidly and severely compromised by over nutrition, particularly on a diet high in saturated fat, which induces inflammation involving both the IKKβ/NF-κB and JNK inflammatory pathways coupled with oxidative and ER stress and the autophagy defect. All of these pathways and processes are interlinked and can be considered causal in the loss of central insulin and leptin signalling, and a prerequisite for the development of obesity. Also, the hypothalamus is a highly plastic tissue undergoing constant neuronal and synaptic remodelling in response to nutrient and hormonal signalling with astrocytes and microglia playing important roles in these processes. In response to obesity and a high-fat diet, a reactive gliosis occurs compromising the structure and the function of the hypothalamus. The paradox of how diet-induced hypothalamic inflammation results in obesity while the sickness-induced inflammation results in anorexia, even though the same inflammatory pathways are the basis for both, must surely depend on the nutritional background and the role of lipids in hypothalamic neuronal signalling.

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