Lipid sensing in the brain and regulation of energy balance


a CNRS EAC 4413, biologie fonctionnelle et adaptative, Paris, France
b Université Paris Diderot, Paris, France
c Neurology Service, VA Medical Center, East Orange, NJ, USA, Department of Neurology and Neurosciences, Rutgers-NJ Medical School, Newark, NJ, USA

Abstract

Nutrient-sensitive neurons [to glucose and fatty acids (FAs)] are present at many sites throughout the brain, including the hypothalamus and brain stem, and play a key role in the neural control of energy and glucose homeostasis. Through their neuronal output, FAs can modulate feeding behaviour as well as insulin secretion and activity. Central administration of oleate, for example, inhibits food intake and glucose production in rats. This suggests that daily variations in plasma FA concentrations could be detected by the central nervous system as a signal that contributes to regulation of energy balance. At the cellular level, subpopulations of neurons in the ventromedial and arcuate hypothalamic nuclei are selectively either inhibited or activated by FAs. Possible molecular effectors of these FA effects most likely include the chloride and potassium ion channels. While intracellular metabolism and activation of the ATP-sensitive K⁺ channels appear to be necessary for some signalling effects of FAs, at least half the FA responses in ventromedial hypothalamic neurons are mediated by interaction with fatty acid translocase (FAT)/CD36, an FA transporter/receptor that does not require intracellular metabolism to activate downstream signalling. Thus, FAs and their metabolites can modulate neuronal activity by directly monitoring the ongoing fuel availability for brain nutrient-sensing neurons involved in the regulation of energy and glucose homeostasis. Besides these physiological effects, FA overload or metabolic dysfunction may also impair neural control of energy homeostasis and contribute to obesity and/or type 2 diabetes in predisposed subjects.

Keywords: Hypothalamus; FAT/CD36; Potassium channel; Energy balance

1. Introduction

The central nervous system (CNS) is a key player in the regulation of energy balance in mammals [1]. The process involves a combination of signals arising from the periphery, including hormones (such as leptin, insulin and ghrelin) and nutrients [glucose and fatty acids (FAs)], detected by specialized brain areas like the hypothalamus and brain stem [2,3]. Since the work by Oomura et al. [4], there is growing evidence suggesting that hypothalamic FA sensing plays a role in the regulation of energy balance, including insulin secretion and activity, hepatic glucose production, linear growth, adipose deposition and food intake [5–8]. The molecular mechanisms involved in this FA sensing by the brain are still a matter of debate, but they may well include plasma membrane proteins such as fatty acid translocase (FAT)/CD36 as well as intracellular events involving acyl-coenzyme A (CoA) synthase and FA oxidation [9]. In addition, recent studies have highlighted the role of neuronal lipoprotein lipase (LPL)-mediated hydrolysis of triglyceride (TG)-enriched particles in the regulation of energy balance [10].

The present report is a review of the mechanisms of lipid actions in CNS areas controlling energy homeostasis (with a focus on the hypothalamus) at molecular, cellular and systems levels under physiological conditions. Furthermore, deregulation of brain FA sensing may contribute to further deterioration of energy balance and ultimately to obesity, with or without type 2 diabetes as a complicating factor [11]. A better understanding of these mechanisms, and further characterization of FA-sensitive neurons and their role in physiological and pathological processes could lead to the identification of novel pharmacological targets for the prevention and treatment of diabetes and obesity.
2. Transport of FAs to the brain and neurons

Cerebral lipids are an essential component of both membranes and intracellular signalling pathways. They represent 50% of brain dry weight, the highest organ lipid content after adipose tissue [12,13]. A growing body of evidence suggests that cerebral lipids are derived by both local synthesis and uptake from the blood circulation. Several studies show that some polyunsaturated FAs (PUFAs) have the ability to cross the blood–brain barrier (BBB) [14,15]. Once across the BBB, it is likely that neurons then take up FAs, as some neurons appear to have FA transporters. Dissociated neurons from the hypothalamic ventromedial nucleus (VMN) of rats, for example, express mRNA for FA transport proteins (FATP)-1 and -4 and for the FAT transporter/receptor FAT/CD36 [7,16]. In addition, while it is unlikely that neurons derive much of their energy supply from FAs, these same neurons express mRNA for intracellular FA metabolism such as long-chain acyl-CoA synthase (ACS), carnitine palmitoyltransferase-1A and -1C (CPT1A/1C), and uncoupling protein 2 (UCP2) [16]. They also express enzymes for de novo FA synthesis such as FA synthase (FAS) [16]. However, it is likely that much of the reported oxidation of FAs such as palmitate in the brain probably takes place in astrocytes [17], whereas other FAs such as arachidonate are largely incorporated into phospholipids [14]. Interestingly, lipoprotein lipase (LPL) has recently been demonstrated to play a role in the regulation of energy balance by neurons [18]. The role of LPL in the brain is to convert triglyceride (TG)-rich lipoproteins into FA locally, thereby providing an indication of the metabolic state to FA-sensitive neurons [10].

3. Some hypothalamic neurons are lipid-responsive

Over the past decade, there has been growing evidence to demonstrate that hypothalamic FA sensing is critical for the regulation of food intake as well as insulin secretion, hepatic glucose production and lipogenesis [9]. A 6-h intracerebroventricular (ICV) infusion of the monounsaturated FA oleic acid (OA) reduced both food intake and hepatic glucose production (HGP) [8], while reducing hypothalamic FA oxidation by inhibition of CPT1 mimicked the effects on food intake and HGP induced by ICV infusion of OA [19]. In another study, direct bilateral infusion of OA into the mediobasal hypothalamus decreased HGP [20]. However, ICV and direct infusions of FA into the brain are not physiological. Interfering with FA oxidation is more likely to have a major effect on astrocyte than neuronal metabolism [21]. They could even produce non-specific effects by evoking an inflammatory response by irritating the ependymocytes and tanyocytes lining the ventricles or exciting the microglia and astrocytes in the brain parenchyma.

Other, more physiological routes include raising systemic levels of FAs or infusing them directly into the carotid arteries, the primary route by which FAs reach the forebrain. In fact, a two-fold increase in plasma TG produced by a 2-day systemic infusion of TG emulsion was associated with decreased sympathetic activity [22]. The reduced sympathetic tone, which can also result from central FA infusions [22], could contribute to the associated FA-induced exaggeration of glucose-induced insulin secretion (GIIS), a condition similar to what happens in the prediabetic state [22]. In addition, this exaggerated GIIS and reduction in HGP have been mimicked by infusing TG into the carotid artery [5], but were decreased by central inhibition of CPT1 [22]. Similarly, central inhibition of CPT1 has been associated with an increase in the acyl-CoA intracellular pool postulated to be the ‘final’ satiety signal rather than the FAs themselves [23].

Nevertheless, there are at least two potential problems involved in the interpretation of such data in vivo. First, the idea that increases in brain FA levels act as a satiety signal to inhibit feeding [19] is counter-intuitive, given the fact that plasma FA levels do not rise substantially after food ingestion, but instead rise significantly during fasting, a setting in which food intake would be expected to increase [24]. Second, the vast majority of FA oxidation in the brain takes place in astrocytes rather than neurons [17]. While a selected group of neurons in the hypothalamus clearly respond directly to changes in ambient FA levels by altering their activity [4,7,16], only a relatively small percentage of these responses depend on neuronal FA metabolism [16]. Furthermore, although β-oxidation and the formation of malonyl-CoA and FA metabolites such as acyl-CoA may be mediators of the effects produced by FA infusions in vivo [25], it is likely that these mostly occur at the level of the astrocyte. If so, then there must be a mechanism by which alterations in astrocyte FA metabolism can provide a signal to those neurons that regulate HGP and food intake. Thus, it may be proposed that the communication between astrocyte FA metabolism and neuronal FA sensing involves the production and export of ketone bodies from astrocytes and their subsequent uptake by neurons [17].

4. Molecular mechanisms of neuronal FA sensing

In FA-sensitive neurons, exposure to long-chain FAs can alter the activity of a wide variety of ion channels such as Cl⁻, GABA_A [26], potassium, K⁺-Ca²⁺ [27] and calcium channels [28]. OA activates arcuate nucleus proopiomelanocortin (ARC POMC) neurons by inhibiting ATP-sensitive K⁺ (K_ATP) channel activity, and the effect of OA on HGP is abolished by ICV administration of a K_ATP-channel inhibitor [29]. Using electrophysiological approaches in vivo and in vitro, OA-sensitive neurons have been characterized using whole-cell patch-clamp recordings in ARC slices from 14- to 21-day-old rats [30]. In these experiments, the vehicle was artificial cerebrospinal fluid (aCSF) with added β-cyclodextrin (complexed with OA) [30]. In these neurons, 13% were excited by OA and 30% were inhibited by OA [30]. The excitatory effects of OA appeared to be due to the closure of chloride channels, thus leading to membrane depolarization and increased action potential frequency [30]. On the other hand, OA inhibitory effects might involve the K_ATP channels as such inhibition was reversed by the K_ATP-channel blocker tolbutamide [30]. Using fura-2 Ca²⁺ imaging of dissociated neurons from the VMN, it was found that OA excited up to 43%, and inhibited up to 29%, of all VMN neurons independent of glucose concentrations [16]. However, in these neurons, inhibition of the K_ATP channel mediated FA sensing in only a
5. Metabolic-dependent FA-sensing effects

Some of the effects of FAs on the activity of some neurons are dependent upon intracellular FA metabolism. Enzymes involved in FA metabolism, such as FAS, CPT1 and acetyl-CoA carboxylase (ACC), are expressed in some hypothalamic neurons as well as in glial cells [16]. Malonyl-CoA may be an important sensor of energy levels in the hypothalamus. It is derived from either glucose or FA metabolism via glycolysis or β-oxidation, respectively. The steady-state level of malonyl-CoA is determined by its rate of synthesis catalyzed by ACC relative to its rate of turnover catalyzed by FAS. Synthesis of malonyl-CoA is the first committed step of FA synthesis, and ACC is the major enzyme of regulation in that process. This means that when the supply of glucose is increased, malonyl-CoA levels increase in keeping with the reduced need for FA oxidation. This increase in both malonyl-CoA and acetyl-CoA levels is associated with reduced food intake.

Central administration of C75, an inhibitor of FAS, also increases malonyl-CoA concentration in the hypothalamus, suppresses food intake and leads to profound weight loss [52]. It has been proposed that, centrally, C75 and cerululin (another inhibitor of FAS) can alter the expression profiles of feeding-related neuropeptides often by inhibiting the expression of orexigenic peptides such as neuropeptide Y [33]. Whether through centrally mediated or peripheral mechanisms, C75 also increases energy expenditure, which contributes to weight loss [34,35]. Studies in vitro and in vivo have demonstrated that at least part of the C75 effects are mediated by modulation of AMP kinase, a known energy-sensing kinase [36]. Indeed, ICV administration of 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), a 5′-AMP-kinase activator, rapidly lowers hypothalamic malonyl-CoA concentration while increasing food intake [35].

These effects correlate closely with the phosphorylation-induced inactivation of ACC, an established target of AMP-activated protein kinase (AMPK). Collectively, these data suggest a role for FA metabolism in the perception and regulation of energy balance. However, it should be pointed out that C75 and AICAR may also have non-specific or even opposite effects, and that the regulation of FA oxidation rate in hypothalamic cells (astrocytes and neurons) by AMPK has not yet been demonstrated. A major effect of C75 is to activate CPT1 rather than cause its inhibition in vitro [37]. Furthermore, the route of FA administration is a critical point. In the above-mentioned study, rats were treated with bolus ICV injections of FAs, which could have deleterious effects due to irritation of ependymocytes and tanyocytes. Again, however, because so much of FA metabolism takes place in astrocytes, all manipulations done in vivo and in slice preparations are likely to alter FA metabolism in astrocytes, and could lead to their primary effects by the production of ketones [16] that could then be used by neurons to alter their FA and glucose sensing [17].

6. Non-metabolic-dependent neuronal FA sensing

While intracellular FA metabolism may be responsible for altering the activity of some FA-sensitive neurons such as ARC POMC neurons [29], it accounts for a relatively small proportion of OA effects on dissociated VMN neurons [7,16]. In the latter neurons, CPT1 inhibition, reactive oxygen species (ROS) formation, long-chain acyl-CoA synthetase and K<sub>ATP</sub>-channel activity, and UCP2 activation account for no more than 20% of the excitatory, and approximately 40% of the inhibitory, effects of OA [16]. In contrast, inhibition of FAT/CD36, an FA transporter/receptor that alters cell function independently of intracellular FA metabolism, reduces OA excitatory and inhibitory effects by up to 77% in FA-excited neurons, with complete inhibition of FA-inhibited neurons that are also glucose-sensitive [7,16]. This means that, in most VMN FA-sensing neurons, CD36 can act primarily as a receptor rather than a transporter of long-chain FA, as it does in taste cells on the tongue, where it activates store-operated calcium channels to alter membrane potential and serotonin release [38]. These effects all arise in the presence of nanomolar concentrations of OA in dissociated neurons [16], whereas micromolar concentrations are generally required to effect similar changes in neuronal activity in brain slice preparations [29–31].

This means that, in the absence of astrocytes, OA can directly affect VMN neuronal activity through both metabolic and non-metabolic pathways. Alternatively, FAs may act as signaling molecules via covalent attachment to proteins (N-terminal acetylation) to alter the function of membrane and intracellular signaling molecules. Palmitoylation, for example, facilitates the targeting and plasma-membrane binding of proteins that otherwise would remain in the cytosolic compartment [39]. Some membrane proteins [such as transforming growth factor alpha (TGFα), synaptosomal-associated 25-kDa protein (required for exocytosis) and plasma-membrane receptors (seven transmembrane receptors such as α<sub>2a</sub>- and β<sub>2</sub>-adrenoceptors) are typically palmitoylated on one or several cysteine residues located adjacent to or just within the transmembrane domain [39]. Such mechanisms might also modulate neuronal FA sensing.

While FAs such as OA and linoleic acid can depolarize neurons, others may in fact impede signaling and neuronal function. Atypical protein kinase C (PKCθ) is expressed in discrete neuronal populations in the ARC and dorsomedial hypothalamic nucleus [40]. CNS exposure to palmitic acid (PA) via direct infusion or by oral gavage increases the localization of PKCθ to hypothalamic cell membranes in association with impaired hypothalamic insulin and leptin signaling [40]. This finding was specific to PA, as the monounsaturated FA OA neither increased membrane localization of PKCθ nor reduced insulin signaling.
Furthermore, ARC-specific knockdown of PKCα attenuated diet-induced obesity and improved hypothalamic insulin signaling. These results suggest that many of the deleterious effects of high-fat diets, specifically those enriched with PA, are CNS-mediated via PKCα activation, resulting in reduced insulin activity.

7. Which neurotransmitters and neuropeptides?

The ultimate consequence of the activation or inactivation of a neuron is the release of neurotransmitters and neuropeptides. As FAs decrease food intake, they might be expected to alter the activity of neurons specifically involved in the regulation of feeding. In fact, OA activates catabolic POMC neurons directly, apparently via β-oxidation and inactivation of the KATP channel in hypothalamic slice preparations [29]. Obici et al. [19] reported that IVC OA administration in vivo markedly inhibited glucose production and food intake, accompanied by a decrease in hypothalamic expression of anabolic neuropeptide Y. Similarly, reduction of ventromedial hypothalamic (VMH) CD36 expression was associated with reduced expression of both orexigenic agouti-related protein (AgRP) and POMC in association with redistribution of fat from visceral to subcutaneous depots and marked impairment of insulin sensitivity [7,16,31]. In contrast, an omega-3 FA-enriched diet increased food intake in anorexic tumour-bearing rats together with decreases in tumour appearances, tumour growth and onset of anorexia [41]. In these treated rats, neuropeptide Y immunoreactivity increased by 38% in the ARC and 50% in the paraventricular nucleus, whereas α-melanocyte-stimulating hormone (a catabolic peptide cleavage product of POMC) decreased by 64% and 29% at these sites, respectively [41].

8. Pathological implications of excess FAs

Given the physiological regulation of energy balance by hypothalamic neuronal FA sensing, impaired regulation of such sensing may then contribute to the development of metabolic diseases such as obesity and type 2 diabetes in predisposed subjects exposed to chronic lipid overload [9]. Also, excess brain lipid levels may alter the control of glucose and lipid homoeostasis through changes in autonomic nervous system activity. Indeed, increasing brain FA levels reduces sympathetic activity and increases GIIIS in rats [8,42], a condition that can exacerbate the development of type 2 diabetes. Furthermore, lipid overload due to a high-fat diet may alter both hypothalamic monoamine turnover [43] and peripheral sympathetic activity in rats [44]. In humans, overweight is often associated with altered sympathetic tone, suggesting a relationship between lipids and autonomic control centres in the brain [45,46].

9. Conclusion

There is increasing evidence that specialized neurons within the hypothalamus and other brain regions, such as the brain stem and hippocampus, can detect changes in plasma FA levels by having FAs directly or indirectly alter the FA-sensitive neurons involved in the regulation of energy and glucose homoeostasis. The neuronal networks of these FA-sensitive neurons that sense and respond to FAs are probably highly complex, given the fact that FAs can both inhibit and excite specific neurons. In addition, many of these neurons use glucose as a signalling molecule, and there is often an inverse responsiveness of such ‘metabolic-sensing’ neurons to FAs vs. glucose. Thus, these neurons are ideally suited to respond differentially in a variety of metabolic conditions such as fasting, feeding and hypoglycaemia. However, while it is clear that specific neurons can respond to changes in ambient FA levels, many questions still remain. As yet, it is not known for certain how FAs are transported to the brain, astrocytes and neurons or whether the FAs that are transported are derived from circulating free FAs or TG. As most studies suggest that rising FA levels reduce food intake, it is now necessary to explain why plasma FA levels are most elevated during fasting, when the drive to seek and ingest food should be at its strongest. Another major issue is related to the interaction between astrocytes and neurons in FA metabolism and signalling. Finally, little is currently known of the basic mechanisms used by neurons to sense FAs, of where such FA-sensitive neurons reside in the brain and what neurotransmitters/neuropeptides they release when responding to FAs.

It has been postulated that diabetes may be a brain disorder [47]. If so, dysfunction of these FA-sensitive neurons might be, at least in part, among the early mechanisms underlying an impaired neural control of energy and glucose homoeostasis and the development of obesity and type 2 diabetes in predisposed subjects. A better understanding of this central nutrient-sensing capacity, including both FAs and glucose, could provide clues towards identifying new therapeutic targets for the prevention and treatment of both diabetes and obesity.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgement

This work was supported by a research fellowship (C.M.) from the French Diabetes Association—ANTADIR (National Association for the Home Treatment of Respiratory Failure) 2010. Alexandre Picard received a PhD grant from CORDDIM (major areas of interest are cardiovascular, obesity, kidney and diabetes).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.diabet.2013.10.001.

References


