Brain lipid sensing and nervous control of energy balance

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Abstract
Nutrient sensitive neurons (glucose and fatty acids (FA)) are present in many sites throughout the brain, including the hypothalamus and brainstem, and play a key role in the neural control of energy and glucose homeostasis. Through neuronal output, FA may modulate feeding behaviour as well as both insulin secretion and action. For example, central administration of oleate inhibits food intake and glucose production in rats. This suggests that daily variations in plasma FA concentrations might be detected by the central nervous system as a signal which contributes to the 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 FA. Possible molecular effectors of these FA effects likely include chloride or potassium ion channels. While intracellular metabolism and activation of the ATP-sensitive K+ channel appear to be necessary for some of the signaling effects of FA, at least half of the FA responses in ventromedial hypothalamic neurons are mediated by interaction with FAT/CD36, a FA transporter/receptor that does not require intracellular metabolism to activate downstream signaling. Thus, FA or their metabolites can modulate neuronal activity as a means of directly monitoring ongoing fuel availability by brain nutrient-sensing neurons involved in the regulation of energy and glucose homeostasis. Besides these physiological effects, FA overload or metabolic dysfunction might 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; Review

Résumé
Détection centrale des acides gras et contrôle de la balance énergétique.

Des neurones sensibles aux nutriments (glucose, acides gras libres [AGL]) ont été localisés dans différentes structures du système nerveux central (SNC), notamment l’hypothalamus et le tronc cérébral, régions clés impliquées dans le contrôle nerveux de la balance énergétique. Sur la base de modèles précliniques, il a été ainsi montré que les AGL participent au contrôle nerveux de la prise alimentaire, ainsi qu’à la sécrétion et à l’action de l’insuline, à la suite de leur effet sur certains neurones hypothalamiques et un relais efférent via le système nerveux autonome. Cela suggère que les variations circadiennes des concentrations circulantes des AGL pourraient être détectées dans des conditions physiologiques par le SNC et contribuer ainsi à la régulation de l’homéostasie énergétique. À l’échelon cellulaire, des sous-populations de neurones « excités » ou « inhibés » par les AGL ont donc été identifiées dans différents noyaux hypothalamiques (arqué ou ventromédian). L’effet activateur ou inhibiteur des AGL implique notamment des canaux chlorures ou potassiques. Le métabolisme intracellulaire des AGL semble être aussi important pour relayer leurs effets mais des données récentes indiquent que dans la moitié au moins des neurones sensibles au AGL, ce soit un mécanisme dépendant du transporteur membranaire des acides gras, FAT/CD36, qui soit impliqué. En conclusion, les AGL ou leurs métabolites ont donc un effet important de régulation de l’homéostasie énergétique via un effet sur les neurones hypothalamiques spécialisés dans la détection des variations quotidiennes des concentrations circulantes des nutriments. À côté de ces aspects physiologiques, une surcharge en lipides ou une dérégulation du métabolisme lipidique pourrait affecter ce système central de détection et consécutivement être un événement précocé pouvant conduire à une détérioration du contrôle nerveux de la balance énergétique. Cela pourrait participer, au moins en partie, au développement des maladies métaboliques (obésité/diabète de type 2) chez des sujets prédisposés.

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Mots clés : Hypothalamus ; FAT/CD36 ; Canaux potassiques ; Balance énergétique ; Revue

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Since free fatty acids (FA) are not a primary metabolic fuel for neurons, their role in brain metabolism has remained questionable. However, accumulating evidence suggests that FA are used in specific areas of central nervous system (CNS), not as nutrients, but as cellular messengers which inform “FA sensitive neurons” about the energy status of the whole body [1–2]. This phenomenon, called “FA sensing”, may be involved in the control of feeding behaviour, hepatic glucose production and insulin secretion. Here, we will review the mechanisms of FA actions on CNS areas controlling energy homeostasis (with a focus on the hypothalamus) at the molecular, cellular and systems levels under physiological and pathophysiological situations. In addition, dysfunction of central FA sensing could be a contributing factor to the early development of type 2 diabetes mellitus and/or obesity which leads to further dysfunction in predisposed subjects. A better understanding of these mechanisms, as well as further characterization of FA sensitive neurons and their role in physiological and pathological processes, might lead to identification of novel pharmacological targets for the prevention and treatment of diabetes and obesity.

1. Transport of FA uptake into the brain and neurons

Cerebral lipids are an essential component of both membranes and intracellular signaling pathways. They represent 50% of brain dry weight; the highest organ lipid content after adipose tissue [3–4]. However, the mechanism by which FA are transported into the brain remains poorly understood. A growing body of evidence suggests that cerebral lipids are derived both from local synthesis and uptake from the blood [5]. Several studies show that some poly-unsaturated FA (PUFA) have the ability to cross the blood-brain barrier (BBB) [5–6]. The question of whether brain FA uptake occurs by passive diffusion or involves a protein which facilitates the transport is still matter of debate. However, once across the BBB, it is likely that neurons can take up FA since some neurons do appear to have FA transporters. For example, dissociated neurons from the hypothalamic ventromedial nucleus of rats express mRNA’s for FA transport proteins (FATP)-1 and 4 and the FA transporter/receptor FAT/CD36 [7]. Also, while it is unlikely that neurons derive much of their energy supply from FA, these same neurons do express mRNA’s for the intracellular metabolism of FA such as long chain acyl-CoA synthetase, carnitine palmitoyltransferase-1a and 1c and uncoupling protein-2 [7]. They also express enzymes for de novo FA synthesis such as FA synthetase [7]. But, it seems likely that much of the reported oxidation of FA such as palmitate in the brain probably occurs in astrocytes [8], whereas other FA such as arachidonate are largely incorporated into phospholipids [5].

2. Some hypothalamic neurons are lipid responsive

The presence of neurons sensitive to variations in extra-cellular glucose levels is clearly demonstrated in the brain and, in particular, in the hypothalamus [1,9–10]. Thirty-five years ago, Oomura et al. first showed that FA activated lateral hypothalamic neurons which suggested a role for FA as neuronal signaling molecules [11]. As shown in Fig. 1, FA also modify neuronal firing rate in hypothalamic arcuate nucleus (ARC) [12]. Such data suggest that physiological variations of plasma FA concentrations (reflecting the metabolic state and energy availability) can be detected and integrated by FA sensing neurons in critical brain areas involved in the regulation of feeding behaviour, glucose and lipid metabolism.

The physiological relevance of brain FA sensing is supported by various studies showing that local increases in brain and hypothalamic FA levels are associated with changes in insulin secretion and hepatic glucose output with variable effects on food intake [13–16]. For example, a 6 h intracerebroventricular (icv) infusion of the monounsaturated FA, oleic acid (OA), reduced food intake as well as hepatic glucose production (HGP) [14]. Reducing hypothalamic FA oxidation by inhibition of carnitine palmitoyl transferase-1 (CPT1), the enzyme that promotes β-oxidation by facilitating the transport of medium- and long-chain FA into mitochondria, mimicked these effects on food intake and HGP induced by icv infusion of OA [17]. In another study, a direct bilateral infusion of OA into the mediobasal hypothalamus decreased HGP [16]. In addition, it seems that the hypothalamus differentially senses FA. For example, icv infusions of OA or docosahexanoic acid, but not palmitic acid, reduce food intake and body weight [15]. However, icv and direct infusions of FA into the brain are not physiological. Thus, they might produce non-specific effects by evoking an inflammatory response by irritating ependymocytes and tanyocytes lining the ventricles or by exciting microglia and astrocytes in the brain parenchyma.

More physiological routes include elevating systemic levels of FA or infusing them directly into the carotid arteries, the major route by which FA reach the forebrain. For example, a two-fold increase in plasma triglycerides produced by a 2 day systemic infusion of triglycerides was associated with decreased sympathetic activity. This reduced sympathetic tone, which is also produced by central FA infusions [18], might contribute to the associated FA-induced exaggeration of glucose-induced insulin secretion (GIIS), a condition which is similar to what occurs in the prediabetic state [18]. Also, this exaggerated GIIS and

![Fig. 1. In vivo multi-unit recording of neuronal activity in arcuate nucleus of rats in response to a single intracarotid injection of saline (A) or oleate (B). Oleate infusion leads to change in frequency of neuronal firing rate.](image-url)
a reduction in HGP were mimicked by infusing triglycerides into the carotid artery [19]. These exaggerated responses were reduced by central inhibition CPT1 [18]. Similarly, central CPT1 inhibition was associated with an increase in the acyl CoA intracellular pool, which was postulated to be the “final” satiety signal rather than FA themselves (review in [9,20]).

However, there are at least two potential problems involved in the interpretation of such in vivo data. First, the idea that increases in brain FA levels act as a satiety signal to inhibit feeding [17] is counterintuitive given the fact that plasma FA levels do not rise substantially after food ingestion, but do rise significantly during fasting [21]. Second, the vast majority of FA oxidation in the brain occurs in astrocytes rather than neurons [8]. While a select group of neurons in the hypothalamus clearly responds directly to changes in ambient FA levels by altering their activity [7,11], only a relatively small percentage of these responses depend upon neuronal FA metabolism [7]. Furthermore, although β-oxidation and formation of malonyl-CoA and FA metabolites such as acyl-CoA may be mediators of the in vivo effects produced by FA infusions [1,22], it is likely that most of these 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, which regulate HGP and food intake. We suggest that this communication between astrocyte FA metabolism and neuronal FA sensing involves the production and export of ketone bodies from astrocytes [8] and subsequent uptake by neurons.

3. Molecular mechanisms involved in neuronal FA sensing

In FA sensitive neurons, exposure to long chain FA can alter the activity of a wide variety of ion channels including Cl−, GABA_A [23], potassium, K+-Ca²⁺ [24] or calcium channels [25]. Additionally, FA inhibit the Na⁺-K⁺ ATPase pump [25]. For example, OA activates ARC POMC neurons by inhibiting ATP-sensitive K⁺ (K_ATP) channel activity [26] and the effect of OA on HGP is abolished by icv administration of a K_ATP channel inhibitor [26]. However, K_ATP channels are ubiquitously expressed on neurons throughout the brain, not only in FA sensing neurons, making the mechanism and site of such in vivo manipulations difficult to discern [27]. Using in vivo and in vitro electrophysiological approaches, OA sensitive-neurons have been characterized using whole cell patch clamp records in ARC slices from 14 to 21 day old rats [12]. Of these, 13% were excited by OA and 30% were inhibited by OA [11]. The excitatory effects of OA appeared to be due to closure of chloride channels leading to membrane depolarization and increased action potential frequency [2]. On the other hand, inhibitory effect of OA may involve the K_ATP channels since this inhibition was reversed by the K_ATP channel blocker tolbutamide [2]. Using fura-2 Ca²⁺ imaging in dissociated neurons from the ventromedial hypothalamic nucleus (VMN) neurons (Fig. 2), we found that OA excited up to 43% and inhibited up to 29% of all VMN neurons independently of glucose concentrations [7]. However, in these neurons, inhibition of the K_ATP channel mediated Fig. 2. Representative changes in intracellular Ca²⁺ concentration ([Ca²⁺]i) oscillations following exposure to incremental concentrations of oleic acid (OA) in freshly dissociated ventromedial nucleus neurons from 3- to 4-wk-old male Sprague-Dawley rats. Neurons were first characterized by their glucosensing category in response to altering glucose concentrations from 2.5 (2.5G) to 0.5 (0.5G) to 2.5 mM (2.5G), followed by 2 or more concentrations of OA. Neurons were tested terminally with 20 nM glutamate (Glut) to ascertain viability. A: glucose-excited/OA-inhibited neuron showing decreased [Ca²⁺]i oscillations at 1 nM and 10 nM OA. B: glucose-inhibited/OA-excited neuron showing increased [Ca²⁺]i oscillations at 1 nM and 10 nM OA. Areas under the curve (AUC) for [Ca²⁺]i oscillations with associated percentage change in responses to alterations in substrate concentrations are shown over each 10-min segment of tracing.

FA sensing in only a small percentage of FA sensing neurons.

Importantly, although a relatively large percentage of hypothalamic neurons are FA sensors, a select population that also sense glucose are highly dependent upon ambient glucose concentrations for the resultant effect of FA on the activity
of these neurons [2,7]. Such data suggest that the responses of hypothalamic FA sensitive neurons are dependent upon the metabolic state of the animal and thus might be expected to respond differently during fasting (when FA levels rise and glucose levels fall) vs. the overfed state when glucose levels rise while free FA levels remain relatively unchanged [2,7]. However, it must be pointed out that FA are naturally complexed to serum albumin in the blood and the concentration of circulating free FA is less than 1% of total FA levels. All the studies investigating FA sensing in the hypothalamus either use non-complexed FA or cyclodextrin-complexed FA in vitro or in vivo. The concentration of free FA in cyclodextrin-complexed FA preparation is unknown. Whether or not the FA concentration used mimicks FA levels in physiological states needs to be determined.

4. Metabolic-dependent FA sensing effects

The effects of FA on activity of some neurons are dependent upon intracellular metabolism of FA. Enzymes involved in FA metabolism such as FA synthase (FAS), CPT1 and acetyl-CoA carboxylase (ACC) are expressed in some hypothalamic neurons as well as in glial cells [1,7]. Malonyl-CoA may be an important sensor of energy levels in the hypothalamus. It is derived from either glucose or FA metabolism via the glycolysis or β-oxidation, respectively. The steady-state level of malonyl-CoA is determined by its rate of synthesis catalysed by ACC relative to oxidation, respectively. The steady-state level of malonyl-CoA is determined by its rate of synthesis catalysed by ACC relative to its rate of turnover catalysed by FAS. The synthesis of malonyl-CoA is the first committed step of FA synthesis and ACC is the major site of regulation in that process. Thus, when the supply of glucose is increased, malonyl CoA levels increase in keeping with a decreased need for FA oxidation. This increase in both malonyl CoA and acyl 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 [28]. It has been proposed that centrally, C75 and cerulenin (another inhibitor of FAS) alter the expression profiles of feeding-related neuropeptides, often inhibiting the expression of orexigenic peptides such as neuropeptide Y [29]. Whether through centrally mediated or peripheral mechanisms, C75 also increases energy expenditure, which contributes to weight loss [30–31]. In vitro and in vivo studies demonstrate that at least part of C75’s effects are mediated by the modulation of AMP-activated kinase, a known energy-sensing kinase [32]. Indeed, icv administration of 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), a S’-AMP kinase activator, rapidly lowers hypothalamic malonyl-CoA concentration and increases food intake [31]. These effects correlate closely with the phosphorylation-induced inactivation of ACC, an established target of AMP kinase. Collectively, these data suggest a role for FA metabolism in the perception and regulation of energy balance. However, it must be also pointed out that C75 and AICAR may also have non-specific or even opposite effects. For example, a major effect of C75 is to activate CPT-1 rather than lead to its inhibition in vitro [33]. Finally the route of administration and the type of FA used are also critical. For example, bolus icv injections of OA, but not palmitic acid, reduce food intake and body weight, possibly mediated through POMC/MC4R signaling [15]. Again, such bolus icv injections could cause non-specific effects related to inflammation of ependymocytes and tanycytes. Also because so much of FA metabolism takes place in astrocytes, such manipulations done in vivo and in slice preparations are likely to alter FA metabolism that takes place in astrocytes which could then indirectly alter neuronal FA sensing [8].

5. Non metabolic-dependent neuronal FA sensing

While intracellular FA metabolism may be responsible for altering neuronal activity in some FA sensitive neurons such as ARC POMC neurons [26], it accounts for a relatively small percent of the effects of OA on dissociated VMN neurons [7]. In those neurons, inhibition of CPT1, reactive oxygen species formation, long-chain acyl CoA synthetase and KATP channel activity or activation of uncoupling protein 2 (UCP2) accounts for no more than 20% of the excitatory or approximately 40% of the inhibitory effects of OA [7]. On the other hand, pharmacological inhibition of FAT/CD36, a FA transporter/receptor that can alter cell function independently of intracellular FA metabolism reduced the excitatory and inhibitory effects of OA by up to 45% [7]. Thus, in almost half of VMN FA sensing neurons, CD36 may act primarily as receptor, rather than a transporter, for long chain FA as it does on taste cells on the tongue where it activates store-operated calcium channels to alter membrane potential and release of serotonin [34]. These effects all occur in the presence of nanomolar concentrations of OA, whereas micromolar concentrations are generally required to effect similar changes in neuronal activity in brain slice preparations [2,12,26]. Thus, in the absence of astrocytes, OA can directly affect VMN neuronal activity through both metabolic and non-metabolic pathways. Alternatively, FA might act as signaling molecules by covalent attachment to proteins (N-terminal acylation) to alter the function of membrane and intracellular signaling molecules. For example, palmitoylation facilitates the targeting and plasma membrane binding of proteins which otherwise would remain in the cytosolic compartment [35]. Some membrane proteins (TGFα, synaptosomal associated protein of 25KDa (required for exocytosis) and plasma membrane receptors (seven transmembrane receptors such as α2a- and β2-adrenoceptors) are typically palmitoylated on one or several cysteine residues located adjacent to or just within the transmembrane domain [35]. Such mechanisms might also modulate neuronal FA sensing.

Finally, while FA such as OA and linoleic acid can depolarize neurons, others may actually impede signaling and neuronal function. For example, the atypical protein kinase C, PKCθ, is expressed in discrete neuronal populations of the ARC and the dorsal medial hypothalamic nucleus [36]. CNS exposure to palmitic acid via direct infusion or by oral gavage increased the localization of PKCθ to hypothalamic cell membranes in association impaired hypothalamic insulin and leptin signaling [36]. This finding was specific for palmitic acid, as the monounsaturated FA, OA, neither increased membrane localization of PKCθ nor reduced insulin signaling. Finally,
ARC-specific knockdown of PKCθ 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 palmitic acid, are CNS mediated via PKCθ activation, resulting in reduced insulin activity.

6. Which neurotransmitters or neuropeptides?

The ultimate consequence of the activation or inactivation of a neuron is the release of neurotransmitters and neuropeptides. Since FA decrease food intake, they might be expected to alter activity neurons specifically involved in the regulation of feeding. In fact, OA activates catabolic POMC neurons directly, apparently via β-oxidation and inactivation of the K_ATP channel in hypothalamic slice preparations [26]. In vivo, Obici et al. [17] reported that ivc administration of OA markedly inhibits glucose production and food intake, accompanied by a decrease in the hypothalamic expression of the anabolic peptide, neuropeptide Y. This decrease in the expression of such a critical anabolic peptide might contribute to the reduced food intake associated with direct central administration of OA. On the other hand, an n-3 FA enriched diet increases food intake in anorexic tumor-bearing rats, in association with reduced tumor appearance, tumor growth and onset of anorexia [37]. In these treated rats, neuropeptide Y immunoreactivity increased 38% in ARC and 50% in paraventricular nucleus, whereas α-melanocyte stimulating hormone (a catabolic peptide cleavage product of POMC) decreased 64% in the ARC and 29% in the paraventricular nucleus [38]. Finally, in the hippocampus, docosahexaenoic acid (22:6(n-3) increased the spontaneous release of acetylcholine [39].

7. Pathological implications of excess FA

Besides physiological regulation of energy balance by hypothalamic neuronal FA sensing, impaired regulation of such sensing might contribute to the development of metabolic diseases such as obesity and type 2 diabetes in predisposed subjects exposed to a chronic lipid overload [1,9]. Excessive brain lipid levels may indeed alter control of glucose and lipid homeostasis through changes of autonomic nervous system activity. Increasing brain FA levels reduces sympathetic activity and increases GIIS in rats [13,17], a condition which would exacerbate the development of type 2 diabetes mellitus. Also, a lipid overload due to high-fat diet intake alters both hypothalamic monoamine turnover [40] and peripheral sympathetic activity in rats [41]. In humans, overweight is often associated with an altered sympathetic tone [42] suggesting a relationship between lipids and autonomic control centers in brain.

8. Conclusion

In conclusion, there is now increasing evidence that specialized neurons within hypothalamus and other areas such as the brainstem or hippocampus can detect changes in plasma FA levels by having FA directly or indirectly alter the FA sensitive neurons involved in the regulation of energy and glucose homeostasis. The neuronal networks of these FA sensitive neurons that sense and respond to FA are likely very complex given the fact that FA can either inhibit or excite specific neurons. In addition, many of these neurons also utilize glucose as a signaling molecule and there is often an inverse responsiveness of such “metabolic sensing” neurons to FA vs. glucose. Thus, these neurons are ideally suited to respond differentially under a variety of metabolic conditions such as fasting, feeding, hypo- or hyperglycemia. However, while it is clear that specific neurons can respond to changes in ambient FA levels, many questions remain. We still do not know for certain how FA are transported into the brain, astrocytes or neurons and whether those FA that are transported are derived from circulating free FA or triglycerides. Since most studies suggest that rising FA levels reduce food intake, then we must 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 relates to the interaction between astrocytes and neurons with regard to the metabolism and signaling of FA. Also, we still know little about the basic mechanisms utilized by neurons to sense FA, where such FA sensitive neurons reside throughout the brain and what neurotransmitters and peptides they release when responding to FA.

Finally, it has been postulated that diabetes may be a disorder of the brain [43]. If so, dysfunction of these FA sensitive neurons could be, at least in part, one of the early mechanisms underlying impairment of neural control of energy and glucose homeostasis and the development of obesity and type 2 diabetes in predisposed subjects. A better understanding of this central nutrient sensing, including both FA and glucose, could provide clues for the identification of new therapeutic targets for the prevention and treatment of both diabetes and obesity.

Conflict of interest statement

None.

References


