Nutrient sensitive neurons (glucose and fatty acids, FA) are present in both the hypothalamus and the brainstem and play a key role in nervous control of energy homeostasis. Through neuronal output, especially the autonomic nervous system, it is now evidenced that FA may modulate food behaviour and both insulin secretion and action. For example, central administration of oleate inhibits both food intake and hepatic glucose production in rats. This suggests that a slight increase in plasma FA concentrations in the postprandial state might be detected by the central nervous system as a satiety signal. At cellular levels, subpopulations of FA-sensitive neurons (either excited or inhibited by FA) are now identified within the hypothalamus. However molecular effectors of FA effects remain unclear. They probably include ionic channels such as chloride or potassium. FA metabolism seems also required to induce neuronal response. Thus, FA per se or their metabolites modulate neuronal activity, as a mean of directly monitoring ongoing fuel availability by CNS nutrient-sensing neurons involved in the regulation of insulin secretion. Beside these physiological effects, FA overload or dysfunction of their metabolism could impair nervous control of energy homeostasis and contribute to development of obesity and/or type 2 diabetes in predisposed subjects.

© 2007 Elsevier Masson SAS. All rights reserved.

Mots clés : Acides gras ; Système nerveux central ; Homéostasie énergétique ; Détection des nutriments ; Hypothalamus ; Revue

* Corresponding author.
E-mail address: cruciani@paris7.jussieu.fr (C. Cruciani-Guglielmacci).

1262-3636/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.diabet.2007.01.006
1. Fatty acids are able to reach the brain and act as informative molecules

Since free fatty acids (FA) are not fuel for neurons, it was thought for a long time that they could not cross the blood–brain barrier. However, a lot of studies clearly show that FA are used in central nervous system (CNS), not as a nutrient, but as a cellular messenger which informs neurons about the energy homeostasis of the whole body. This phenomenon is called ‘FA sensing’. Thus, a growing amount of evidence shows that FA act in CNS and are involved in the control of feeding behaviour, hepatic glucose production (HGP) and insulin secretion.

We aimed here at reviewing the mechanisms of FA actions on CNS areas controlling energy homeostasis (especially hypothalamus), at molecular, cellular and integrated levels, and in physiological as well as extra-physiological situations. Indeed a dysfunction of FA metabolism could be an early event in type 2 diabetes aetiology thus leading to further dysfunction in predisposed subjects. The understanding of these mechanisms as well as the characterisation of ‘FA responsive’ neurons could be a challenging way of finding new pharmacological targets.

2. FA uptake towards brain

Cerebral lipids are an essential component of both membranes and intracellular signalling pathways. They represent 50% of the brain dry weight, which is the highest lipid content after adipose tissue [1]. FA role as an informative molecule acting on brain has been poorly studied in the past, as they were thought unable to cross the blood–brain barrier. However, a growing amount of data attest that cerebral lipids come from both local synthesis and plasma origin [2]. Near the hypothalamus and in particular the arcuate nucleus (the most external hypothalamic nucleus), the blood–brain barrier is discontinuous, allowing a direct access for hormones and nutrients to the CNS. Even when the blood–brain barrier is present, it has been showed that poly-unsaturated fatty acids (PUFA) have the ability to cross it [1,3], as well as palmitic acid [4]. The question whether FA uptake mechanism is passive diffusion or involves a protein which facilitates the transport (such as fatty acid transport protein 1, FATP-1) is still matter of debate. In addition, we could precise that, once integrated to the acyl-CoA intracellular pool following carnitine palmitoyl transferase-1 inhibition (CPT1, the enzyme allowing medium- and long-chain FA to enter the mitochondria where they undergo β-oxidation), the increased acyl-CoA intracellular pool would be the ‘final’ satiety signal, and not the FA by themselves (review in [14]).

3. Some hypothalamic neurons are lipid responsive

The presence of neurons sensitive to variations of extracellular glucose levels is clearly demonstrated in brain and in particular in the hypothalamus (review in [6,7]). In 1975, Oomura et al. [8] demonstrated that FA activated lateral hypothalamic neurons thus suggesting a role as informative molecules. As shown in Fig. 1, FA also modify neuronal firing rate in hypothalamic arcuate nucleus [9]. Thus, physiological variations of FA plasma concentration (reflecting the metabolic state and energy availability) could be detected and integrated at central level and in turn, involved a regulation of both glucose and lipid metabolism, including feeding behaviour or HGP.

Physiological relevance of this FA sensing is evidenced by various studies. For example, a local increase of FA in brain triggered changes in insulin secretion and action (in particular the hepatic glucose output), with or without food intake modifications [10,11]. These changes appeared to be due, at least in part, to modifications of autonomic nervous system (ANS) activity and more precisely to fluctuations of the sympathovagal tone [12]. Indeed the two antagonist parts of ANS—sympathetic nervous system and parasympathetic nervous system—innerve the pancreas and insulin target tissues, exerting catabolic and anabolic effects, respectively.

Luciano Rossetti group has been studying for years the physiological effects of FA on brain. Thus, they demonstrated that 6 hours of oleic acid (OA) intracerebroventricular (ICV) infusions, exerting catabolic and anabolic effects, respectively.

According to these authors, the ICV infusion of OA mimics the central rise in FA following food ingestion and acts as a satiety signal [11]. However, increased in FA concentration is not observed after food ingestion, whereas fasted-state is a physiological situation in which plasma FA levels increase. These effects on food intake and HGP are also obtained by inhibiting hypothalamic FA oxidation without any FA infusion [13]. Indeed the authors reported an increase in acyl-CoA intracellular pool following carnitine palmitoyl transferase-1 inhibition (CPT1, the enzyme allowing medium- and long-chain FA to enter the mitochondria where they undergo β-oxidation). The increased acyl-CoA intracellular pool would be the ‘final’ satiety signal, and not the FA by themselves (review in [14]).

The regulation of energy metabolism by FA effects on CNS is physiological, however deregulation of FA sensing could lead to FA dysregulation, the hallmark of type 2 diabetes. A decrease in FA uptake towards brain could stimulate excessive HGP and food intake modifications [10,11]. These changes appeared to be due, at least in part, to modifications of autonomic nervous system activity and more precisely to fluctuations of the sympathovagal tone [12]. Indeed the two antagonist parts of ANS—sympathetic nervous system and parasympathetic nervous system—innerve the pancreas and insulin target tissues, exerting catabolic and anabolic effects, respectively.

![Fig. 1. Whole cell current clamp recordings in an OA excited neuron in 2.5 mM glucose in a hypothalamic slice from a 2–3-week-old rat. Resting membrane potential is ~45 mV.](image-url)
partly lead to metabolic diseases in predisposed subjects exposed to a chronic lipid overload. Such impairment of central effects of FA could be involved in the aetiology of obesity and type 2 diabetes [15,16]. Indeed data show that excessive lipid load towards brain may alter nervous control of glucose and lipids homeostasis through changes of ANS activities. First, a lipid overload due to high-fat diet has been demonstrated to be involved in modifications of CNS activities, in rats [17,18]. In humans, overweight is often associated with an altered sympathetic tone [19,20], suggesting a relationship between lipids and ANS control centre in brain. In addition, numerous studies from Pima Indians, a population displaying high prevalence for obesity and diabetes, showed that a decreased sympathetic tone is a predictive feature of further metabolic dysfunction [21].

We have already showed in rats that 2-day systemic infusion of triglycerides—leading to a twofold increase in plasma concentration—induces a decrease in sympathetic nervous activity (Fig. 2). This decrease is responsible, at least in part, for the exaggerated glucose-induced insulin secretion (GIIS) characterising a pre-diabetic state and also observed in these rats [12]. A causal link between a decreased sympathetic tone and the deregulation of insulin secretion has been shown by the use of oxymetazolin, a specific alpha2 adrenergic receptor agonist which normalises GIIS in a dose-dependent manner (Fig. 2 part A). To further study the role of FA on nervous centres controlling glucose homeostasis, we decided to provoke a local increase of lipids in brain. This approach allows us to dissociate direct effects of FA on CNS from other potential mechanisms of afferent counter-regulation. We demonstrated that 2-day ICV infusion of triglycerides lead to an increased GIIS and a hepatic insulin resistance, as well as a lower pancreatic sympathetic tone [10]. The same dysfunctions in insulin secretion and action have been put in evidence in a rat model of intracarotid lipid infusion towards brain [22], a model designed to avoid the by-pass of blood brain barrier. Using a CPT1 inhibitor, we also demonstrated that FA β-oxidation in brain is required for their effects on insulin secretion and action [22] (Fig. 3).

To summarise, it is now clearly admitted that FA can act on CNS to control energy homeostasis. The molecular mechanisms are still unknown, however β-oxidation seems to play a key role and FA metabolites such as acyl-CoA and malonyl-CoA may relay their effects [23].

3.1. Molecular mechanisms involved in FA-sensitive neurons

Most of central area and especially the hypothalamus can detect and respond to plasmatic changes of FA concentrations through the involvement of lipid-sensing neurons. Indeed, these neurons use FA in a concentration-dependent manner as a signalling molecule to regulate their membrane potential and action potential frequency. Thus, FA can modify ion channels activities and consequently induce changes in secretion of neurotransmitters. A body of literature indicates that FA regulate the conductance of a wide variety of ion channels which include Cl−, GABA A [24], CIC-2 [25], K+, K+–Ca2+ [26], K ATP [26] and Ca2+ channels [27]. Additionally, FA inhibit the Na+–K+ ATPase pump [28]. As an example, the degree of FA acylation regulates the K ATP channels activity and Obici et al. evidenced that the effect of OA on HGP is abolished by ICV administration of a K ATP channels inhibitor [29]. However, it must be pointed out that K ATP channels are ubiquitously expressed in the brain—not only in lipid-sensing neurons—and the anorexigenic effect observed in Obici’s experiments could result from a ‘general’ dysfunction of the brain.

![Fig. 2. Illustrative recording segments of sympathetic nerve activity from control (A) and lipid-infused rats (B). The sympathetic nerve, which is close to the carotid artery, was dissected free of underlying tissues over a distance of approximately 1 cm until the superior cervical ganglion. The nerve was then covered with paraffin oil to prevent dehydration and carefully placed on a pair of recording silver wire electrodes (0.6-mm diameter). Electrodes were connected to a high-impedance probe, amplified by 104 with an alternative-current amplifier (time constant 0.2 s) and filtered at low- and high-frequency cut-offs (1–80 kHz). The filtered, amplified signal was routed to a computer for further analysis.](Image)

![Fig. 3. Model of FA sensing neuron within hypothalamus. Increase in malonyl-CoA production (and transient concentration?) leads to CPT1 inhibition, and a consequent decreased β-oxidation and increased intracellular acyl-CoA content. This could be finally a satiety signal. Pharmacological inhibition of CTP1 could also lead to increased acyl-CoA content and both inhibition of food intake and HGP. OAA: oxalo-acetate, FAS: fatty acid synthase, ACC2: acetyl-CoA carboxylase, CPT1: carnitine palmitoyl transferase-1. TG: triglycerides.](Image)
3.2. Roles of cellular metabolites

The cellular and molecular effects of FA are strongly investigated. As already evidenced, FA metabolism seems necessary to relay FA action. It is now established that main enzymes involved in FA metabolism, such as fatty acid synthase (FAS), carnitine palmitoyltransferase-1 (CPT1), acetyl-CoA carboxylase (ACC)… are expressed in both hypothalamic neurons and glial cells. It is clearly suggested that malonyl-CoA is one of the main energy sensors in the hypothalamus (as in skeletal muscle). It results from glucose or FA metabolism, respectively, via the glycolysis or the β-oxidation pathways. 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 of FA synthesis. Malonyl-CoA is an established inhibitor of CPT1, an outer mitochondrial membrane enzyme that controls entry of FA into mitochondria and, thereby, FA oxidation. Thus, when the supply of glucose and FA is increased, malonyl-CoA levels increase in keeping with a decreased need for FA oxidation, and FA are preferentially esterified to produce diacylglycerol and triglycerides. This increase in malonyl-CoA and acyl-CoA levels leads to a strong signal of satiety. Central administration of C75, a potent inhibitor of FAS, also increases malonyl-CoA concentration in the hypothalamus, suppresses food intake and leads to profound weight loss. It has been proposed that centrally, C75 and cerulenin—another inhibitor of FAS—alter the expression profiles of feeding-related neuropeptides (such as NPY), often inhibiting the expression of orexigenic peptides [30]. Whether through centrally mediated or peripheral mechanisms, C75 also increases energy consumption, which contributes to weight loss. In vitro and in vivo studies demonstrate that at least part of C75’s effects are mediated by the modulation of AMP kinase, a known peripheral energy-sensing kinase [31, 32]. Indeed, ICV administration of 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), a 5’-AMP kinase activator, rapidly lowers hypothalamic malonyl-CoA concentration and increases food intake [31]. These effects correlate closely with the phosphorylation and thus 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.

Plasma changes of FA concentrations are responsible for their main effects on neural activity and secretion of neurotransmitters. FA can also play key roles in regulating cellular structure and function through their covalent attachment to proteins. The two most common forms of protein fatty acylation are modification with myristate, a 14-carbon saturated FA, and palmitate, a 16-carbon saturated FA [33,34]. Many N-myristoylated proteins are membrane bound and can be found in the plasma membrane or other intracellular membranes in eukaryotic cells. For example, studies of pp60v-src, the transforming protein of Rous sarcoma virus, have clearly established that myristoylation is necessary for directing the Src protein to the membrane and its binding [34]. Palmitoylation helps to target the membrane and plasma membrane binding of proteins which otherwise would stay in the cytosolic compartment. Some membrane proteins (TGFα, Synaptosomal Associated Protein of 25 kDa (SNAP25, required for the exocytosis pathway…) and plasma membrane receptors (seven transmembrane receptors such as α2A adrenergic receptor, β2 adrenergic receptor…) are typically palmitoylated on one or several cysteine residues located adjacent to or just within the transmembrane domain. Other types of proteins as most α subunits of G proteins are palmitoylated at cysteine residues near the N-or C-termini. Whether acylation with different FA affects protein function has not yet been determined.

Some of FA effects could also be transmitted through their binding to G protein coupled receptors (GPR). For example, OA regulates GIIS from pancreatic β cells through the binding to GPR40 [35]. Immunohistochemical analyses revealed the highest levels of GPR40 mRNA in brain and in insulin-producing pancreatic islets. It has also been evidenced that the obese and insulin-resistant ob/ob mice possessed significantly higher levels of expression of GPR40 mRNA in whole pancreas compared with normal, lean mice. Long-chain free fatty acids (FFAs) are ligands of GPR40, whereas short-chain
carboxylic acids are ligands of GPR41 and GPR43. Analyses of these types of GPR mRNA revealed very different distributions in human tissues. GPR41 revealed highest levels of expression in adipose tissue, while GPR43 showed highest levels in lymphatic tissues. One of our hypotheses is that FA could regulate neurotransmitters secretions through the binding to GPR41, since its mRNA has been detected in the rat hypothalamus (unpublished data).

Finally, it is also established that FA or their metabolites can directly regulate gene expression. Using a micro-array approach, the differential hypothalamic gene expression profile between rats infused ICV with triglycerides and heparin for 48 h and control rats infused with saline and heparin, was studied in our laboratory [36]. Among the hundred genes differently expressed, we identified receptors of neurotransmitters (for example, GABA type A) or proteins involved in different signalling pathways (PKCs).

3.3. Which neurotransmitters?

The ultimate step of the activation/inactivation of a neurone is the release of neurotransmitters. Actually, there is no data that clearly evidence which kind(s) of neurotransmitters is (are) involved in the case of FA-sensitive neurons. As FA decrease food intake, it can be expected that they act through the stimulation/inhibition of anorexigenic (POMC/CART)/orexigenic (NPY/AGRP) neuropeptides expression. Obici et al. [11] reported that ICV administration of OA markedly inhibits glucose production and food intake, accompanied by a decrease in the hypothalamic expression of neuropeptide Y. Recently, it has been evidenced that n-3 FA enriched diet increases food intake in anorexic tumour-bearing rats [37]. Moreover, n-3 FA diet delays tumour appearance, tumour growth, and onset of anorexia. In these treated rats, NPY decreased 64% in ARC and 29% in PVN (P < 0.05). Other data revealed that in the hippocampus, the docosahexaenoic acid (22:6(n-3)) increases the spontaneous release of acetylcholine [38].

4. Conclusion

In conclusion, there is now a growing amount of evidence that specialised neurons within the hypothalamus (and brainstem) could be able to detect changes in plasma FA levels thus contributing to nervous control of energy homeostasis. Such a neuronal network seems very complex since subpopulations of neurons are now identified (either excited or inhibited by FA). Furthermore glucose environment also seems crucial, since either hypo- or hyperglycaemia differently modulate their neuronal activity. Many questions remain without response… For example, what about the neuron/glial cells interactions to modulate this FA sensing? What is their distribution within the hypothalamus and other parts of the brain? Are those FA sensing neurons also sensitive (or not) to glucose? Which neurotransmitters are secreted by FA-sensitive neurons?

Finally, regarding the hypothesis that type 2 diabetes is a disorder of the brain [15], dysfunction of these FA-sensitive neurons could be, at least in part, one of the early mechanisms which could lead to impairment of nervous control of energy homeostasis and to obesity and type 2 diabetes in predisposed subjects. A better understanding of this central nutrient-sensing (including both FA and glucose) could allow to identification of new therapeutic targets.

References


