



# Glutamate and GABA in appetite regulation

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Appetite is regulated by a coordinated interplay between gut, adipose tissue, and brain. A primary site for the regulation of appetite is the hypothalamus where interaction between orexigenic neurons, expressing Neuropeptide Y/Agouti-related protein, and anorexigenic neurons, expressing Pro-opiomelanocortin cocaine/Amphetamine-related transcript, controls energy homeostasis. Within the hypothalamus, several peripheral signals have been shown to modulate the activity of these neurons, including the orexigenic peptide ghrelin and the anorexigenic hormones insulin and leptin. In addition to the accumulated knowledge on neuropeptide signaling, presence and function of amino acid neurotransmitters in key hypothalamic neurons brought a new light into appetite regulation. Therefore, the principal aim of this review will be to describe the current knowledge of the role of amino acid neurotransmitters in the mechanism of neuronal activation during appetite regulation and the associated neuronal-astrocytic metabolic coupling mechanisms. Glutamate and GABA dominate synaptic transmission in the hypothalamus and administration of their receptors agonists into hypothalamic nuclei stimulates feeding. By using  $^{13}\text{C}$  High-Resolution Magic Angle Spinning Nuclear Magnetic Resonance spectroscopy based analysis, the Cerdán group has shown that increased neuronal firing in mice hypothalamus, as triggered by appetite during the feeding-fasting paradigm, may stimulate the use of lactate as neuronal fuel leading to increased astrocytic glucose consumption and glycolysis. Moreover, fasted mice showed increased hypothalamic  $[2-^{13}\text{C}]\text{GABA}$  content, which may be explained by the existence of GABAergic neurons in key appetite regulation hypothalamic nuclei. Interestingly, increased  $[2-^{13}\text{C}]\text{GABA}$  concentration in the hypothalamus of fasted animals appears to result mainly from reduction in GABA metabolizing pathways, rather than increased GABA synthesis by augmented activity of the glutamate-glutamine-GABA cycle.

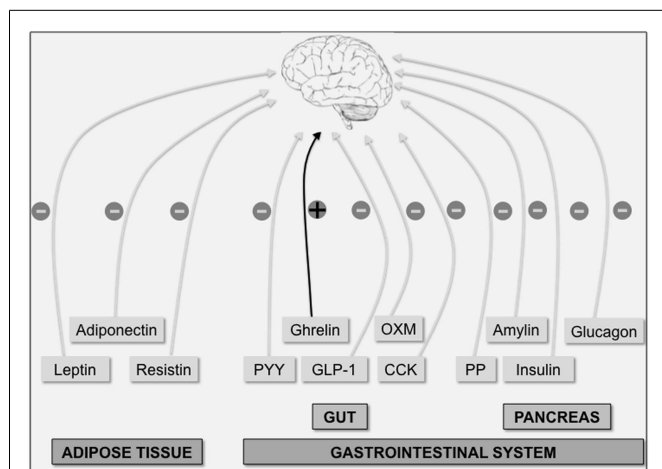
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## APPETITE REGULATION: FROM THE PERIPHERY TO THE HYPOTHALAMUS

Appetite is a highly regulated phenomenon, being hunger and satiety crucial factors in controlling food intake. Both food intake and energy expenditure disturbances lead to obesity, a pandemic syndrome frequently associated with the most prevalent and morbid pathologies in developed countries including heart disease, atherosclerosis, diabetes, and cancer (1). Appetite is closely regulated by a coordinated interplay between peripheral and central nervous system pathways. Two major groups of peripheral-derived signals inform the brain about the whole-body energy state: short-term signals produced by the gastrointestinal system and long-term signals produced by adipose tissue (**Figure 1**). There is a vast array of anorexigenic hormones causing loss of appetite secreted from the gut; these include: cholecystokinin (CCK) (2), glucagon-like peptide-1 (GLP-1) (3), peptide YY (PYY) (4), and oxyntomodulin (OXM) (5). Hormones derived from the pancreas, as pancreatic polypeptide (PP) (6), glucagon (7), insulin (8), and amylin (9), also exhibit anorexigenic actions. Finally, adipose tissue-derived anorexigenic signals, such as leptin (10), adiponectin (11), and resistin (12) have been described. On the other hand, gut-derived ghrelin is the only example of a peripheral hormone with orexigenic actions (13, 14), thereby increasing appetite upon its release

usually before meals. In spite of intensive research during the last decades, other unidentified peripheral signals playing a role in appetite regulation probably exist. An increased knowledge on peripheral inputs controlling appetite could be relevant for the development of newly successful therapeutical approaches targeting obesity.

Studies employing either discrete lesions in the hypothalamus (15, 16) or surgical transection (17) of neural pathways have shown that central integration of peripheral-derived signals occurs mostly in the hypothalamus. The hypothalamus lies adjacent to three circumventricular organs, which are areas that permit substances to leave the brain without disrupting the blood-brain barrier (BBB), thereby permitting other substances that do not cross the BBB to exert their actions in the brain (18). In the last years, several neurotransmitters involved in hypothalamic appetite regulation have been identified [see, for example reviews (19–22)]. The cornerstone experiment for the identification of a potential neurotransmitter consists on the injection of the respective agent into the hypothalamus or adjacent ventricle of animal models and detection of a rapid increase or decrease in food intake. These experiments allowed not only the identification and characterization of several neurotransmitters involved in hypothalamic appetite regulation, but also to the precise tracking of pathways



**FIGURE 1 | The brain integrates multiple peripheral signals to control appetite.** Peripheral factors indicative of long-term energy whole-body status are produced by adipose tissue (leptin, adiponectin, and resistin). On the other hand, acute orexigenic (+) ghrelin signal (produced in the gut) and anorexigenic (–) signals such as the gut hormones peptide YY (PYY), oxyntomodulin (OXM), glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK), and the pancreatic hormones [insulin, glucagon, amylin, and pancreatic polypeptide (PP)] indicate long-term energy status.

containing these signal molecules. Usually, neurotransmitters are classified into peptides, amino acids, and monoamines.

### HYPOTHALAMIC PEPTIDERGIC NEUROTRANSMISSION AND APPETITE REGULATION

In the arcuate nucleus of the hypothalamus, two sets of neuronal populations expressing either orexigenic neuropeptides [Neuropeptide Y (NPY) and Agouti-related peptide (AgRP)], or anorexigenic neuropeptides [Pro-opiomelanocortin (POMC) and Cocaine-amphetamine-related transcript (CART)] co-exist. Neuropeptide Y is synthesized in neurons situated in the far ventromedial aspect of the hypothalamic arcuate nucleus. Within the hypothalamus, NPY-expressing fibers project from the arcuate nucleus to the paraventricular nucleus, where the peptide is released (23). Thus, the administration of NPY to the hypothalamic paraventricular nucleus results in a robust and sustained increase of food intake in rodents (24), eventually leading to obesity when given repeatedly (25). On the other hand, antibody-mediated blockade of NPY action results in decreased food intake in starved animals (26). As neuronal populations expressing NPY are colocalized with AgRP-releasing neurons, the optogenetic (27) or pharmaco-genetic (28) stimulation of AgRP-expressing neurons also drives intense food intake whereas genetic ablation (29, 30) or pharmaco-genetic inhibition (28) decreases food consumption.

Neurons located mainly in the ventrolateral subdivision of the hypothalamic arcuate nucleus contain both the anorexigenic peptide CART and its precursor, POMC. The optogenetic stimulation of POMC-containing neurons reduces food intake (27) whereas genetic ablation of POMC-expressing cells (31, 32) increases appetite and food consumption. The gene encoding POMC gives rise to downstream peptide products, including melanocortins [adrenocorticotrophic hormone (ACTH), the

$\alpha$ -,  $\beta$ -, and  $\gamma$ -melanocyte-stimulating hormones (MSH) and  $\beta$ -endorphin (33). Release of the  $\alpha$ -MSH peptide at the hypothalamic paraventricular nucleus potentially reduces food intake via activation of the melanocortin receptors, MC3R and MC4R. On the contrary, increased food intake and obesity are seen as a result of deletion of MC3R (34) and MC4R (35). In summary, activation of the POMC-expressing neurons in the hypothalamic arcuate nucleus triggers the release of  $\alpha$ -MSH, which activates MC4R at the paraventricular nucleus, leading to suppressed food intake and increased energy expenditure. On the other hand, stimulation of AgRP-expressing neurons in the hypothalamic arcuate nucleus releases AgRP peptide, which antagonizes the effect of  $\alpha$ -MSH on MC4R thereby increasing food intake.

To date, most effort has been placed on examining direct regulation of hypothalamic NPY/AgRP and POMC/CART-expressing neurons by various circulating factors whereas the role of upstream neural inputs has received comparatively less attention. This is surprising considering that both NPY/AgRP and POMC/CART neurons receive abundant excitatory and inhibitory synaptic input. The two neurotransmitters that account for most of the synaptic activity in the hypothalamus are the amino acids glutamate and  $\gamma$ -aminobutyric acid (GABA).

### HYPOTHALAMIC GLUTAMATERGIC NEUROTRANSMISSION NEURONAL-ASTROCYTIC GLUTAMATE METABOLISM

Glutamate is the dominant excitatory neurotransmitter in the central nervous system. In order for a neuron to release glutamate, the neurotransmitter must first be packed at high concentrations into synaptic vesicles, by means of specific vesicular glutamate transporters (VGLUT1, VGLUT2, and VGLUT3) (36). Upon stimulation, glutamate is released into the synaptic cleft to bind and elicit its effects on postsynaptic receptors, whether ionotropic [*N*-methyl-D-aspartate (NMDA), D,L-alpha-amino-3-hydroxy-5-methyl-isoxazole propionic acid (AMPA), kainic acid] or metabotropic receptors (mGluRs), present both in neurons and astrocytes.

Despite of its ubiquitous nature, extracellular glutamate levels are tightly regulated. The release of presynaptic glutamate largely exceeds the amount need for neurotransmission. As high glutamate concentrations could preclude further transmission or become associated with neurotoxicity events unless rapidly cleared, synaptically released glutamate is recycled from the extracellular space by means of excitatory amino acid transporters expressed predominantly on astrocytes (GLT-1 and GLAST). Within astrocytes, recycled glutamate can be metabolized to glutamine via glutamine synthetase or can be assimilated into the tricarboxylic acid (TCA) cycle. Glutamine released from astrocytes is further taken up again by neurons, where the mitochondrial phosphate-specific enzyme, glutaminase, reconverts inert glutamine-to-glutamate for subsequent repackaging into synaptic vesicles: the glutamate-glutamine cycle. Importantly, due to the lack of pyruvate carboxylase in neurons making them incapable of *de novo* synthesis of glutamate from glucose (37), the glutamate-glutamine cycle assures an adequate replenishment of glutamate in the central nervous system (38, 39). However, the glutamate-glutamine cycle faces a drain of compounds by oxidation (40–42), requiring a continuous replenishment of glutamate

and glutamine in astrocytes. *De novo* synthesis of glutamate and glutamine by astrocytes requires an amino group, being that aspartate has been recently suggested as the neuron-born nitrogen donor (43). According to the astrocyte to neuron lactate shuttle hypothesis (ANLSH) (44, 45), energy requirements for astrocyte-mediated glutamate recycling are derived exclusively from the glycolytic glucose metabolism with the concomitant lactate production by astrocytes, the latter becoming the main oxidative fuel for neurons (44, 46, 47).

### GLUTAMATE IN APPETITE REGULATION

The intracerebroventricular injection (48), as well as the lateral hypothalamic injection of glutamate, or its excitatory amino acid agonists, kainic acid, AMPA, and NMDA (49), rapidly elicit an intense food intake in rats. Likewise, intracerebroventricularly injected mGluR5 agonists stimulate feeding in rodents whereas the mGluR5 receptor antagonist (R,S)-2-chloro-5-hydroxyphenylglycine, inhibits food intake (50). Although the above-mentioned studies implicate glutamate signaling in eliciting a stimulation of food intake, until recently the morphological examination of the glutamatergic system was difficult due to the lack of marker molecules specific to glutamatergic neurons. Two highly homologous transmembrane proteins, VGLUT1 and VGLUT2, have been proven to be specific for glutamatergic neurons. On this basis, several studies have identified the presence of a dense plexus of glutamatergic fibers in key hypothalamic areas involved in appetite regulation. For example, elevated expression of mRNA encoding VGLUT2 was found in neurons located in the ventromedial hypothalamus and from the ventrolateral aspect of the arcuate nucleus (51, 52). On the other hand, expression of VGLUT1 is confined to relatively weak labeling in the lateral hypothalamic area (51). Furthermore, by using double-labeling immunohistochemistry, the presence of VGLUT2 immunoreactivity has been shown in appetite-regulating POMC/CART-expressing neurons located in the arcuate nucleus (53, 54), where they receive glutamatergic input from neurons in the ventromedial nucleus of the hypothalamus (55). In addition, Kiss et al. provided evidences for the existence of glutamatergic innervation of NPY-expressing neurons in the rat hypothalamic arcuate nucleus (54).

To evaluate the role of glutamatergic input to NPY/AgRP and POMC/CART-expressing neurons, and more specifically its plasticity as regulated by glutamate NMDA receptors, Liu et al. generated mice lacking NMDA receptors on either AgRP or POMC neurons (56). The authors found that NMDA receptors on AgRP neurons, but not on POMC-expressing neurons, play a critical role in controlling energy balance indicating that fasting-induced activation of AgRP-releasing neurons is associated with markedly increased glutamatergic input (56). Furthermore, through the combination of cell-type-specific electrophysiological, pharmacological, and optogenetic techniques, Yang et al. found that food deprivation elevates excitatory synaptic input. According to these authors, gut-derived ghrelin acts at presynaptic receptors to increase glutamate release and activate NPY/AgRP-expressing neurons through ionotropic glutamate receptors (57).

In the last decade, astrocytes were reported to participate in several neuroendocrine processes although only recently their importance in the control of appetite and energy homeostasis

has been established. Astrocytes express receptors for numerous neuropeptides, neurotransmitters, and growth factors, produce neuroactive substances, and express key enzymes necessary for sensing and processing nutritional signals. For example, the anorexigenic hormone leptin is known to affect astrocyte morphology and synaptic protein levels in the hypothalamus (58). Thereby, the observed diet-induced increase in leptin receptor levels in hypothalamic astrocytes is proposed to participate in the onset of obesity. More recently, Fuente-Martín et al. have shown that leptin directly modulates glutamate uptake in astrocytes in a time-dependent manner, stimulating a rapid increase that is downregulated with chronic exposure (59). The initial rapid increase in astrocyte's glutamate captation indicates that leptin could reduce the stimulatory effects of glutamate at nearby synapses, thereby reducing appetite. In addition, when excess glutamate is released to the synaptic cleft, it is eventually recaptured by surrounding astrocytes, together with sodium ions, through the astrocytic glutamate cotransporter, GLAST. As a result, the intracellular sodium ions incorporated have to be extruded to the extracellular space, through the electrogenic  $\text{Na}^+/\text{K}^+$ ATPase and  $\text{Na}^+\text{K}^+2\text{Cl}^-$  cotransporter, resulting in the intracellular incorporation of potassium ions. Increased intracellular potassium ions concentrations trigger an osmotically driven, aquaporin 4 (AQP4)-mediated, water transport culminating with astrocytic swelling (60). By using diffusion weighting imaging, Lizarbe et al. have recently shown significant increases in the slow diffusion parameters, consistent with astrocyte swelling response, in the hypothalamus of fasted relative to satiated animals (61, 62). On these grounds, we may hypothesize that, whereas an initial leptin-driven glutamate uptake in astrocytes shows anorexigenic potential (by diverting glutamate from neurons and thereby reducing glutamatergic neurotransmission), an excessive glutamate uptake by astrocytes, as occurs under orexigenic fasting conditions, causes astrocyte's swelling and eventual response by amino release to the synaptic cleft (63) (augmenting glutamatergic neurotransmission associated with appetite enhancement).

### HYPOTHALAMIC GABAERGIC NEUROTRANSMISSION NEURONAL-ASTROCYTIC GABAERGIC METABOLISM

$\gamma$ -Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system. The regulation of GABA itself is achieved by several specialized molecular mechanisms mediating transport, sequestration, synthesis, and GABA degradation. GABAergic neurons express both mature isoforms of glutamate decarboxylase, GAD65 and GAD67, to convert the excitatory amino acid glutamate into GABA (64). Moreover, glutamine can be used as an alternative source of GABA. As described in the earlier section, the amino acid glutamine has long been known as the immediate precursor for glutamate. There is increasing evidence for a similar role of this glutamate-glutamine cycle in GABA synthesis [see review (65)]. GABA clearance from the synaptic cleft is mediated by specific, high-affinity, sodium- and chloride-dependent transporters, GAT1, GAT2, and GAT3 and the vesicular GABA transporter (VGAT) (66). After release, GABA elicits a biphasic response via activation of two classes of membrane receptors; either ionotropic ( $\text{GABA}_A$ ) or metabotropic ( $\text{GABA}_B$ ) receptors. Finally, it is estimated that more than 90% of all GABA

in the mammalian central nervous system is degraded by transamination of GABA and  $\alpha$ -ketoglutarate to succinic semialdehyde and glutamate in the mitochondria of astrocytes and neurons (67).

### GABA IN APPETITE REGULATION

A stimulatory role for GABA in the regulation of hypothalamic controlled feeding behavior has been evidenced in the last years. The intracerebroventricular administration of the GABA<sub>A</sub> receptor agonist, muscimol, stimulates feeding in satiated pigs, a response blockable by the specific GABA<sub>A</sub> receptor antagonist, bicuculline (68). Also, systemic and intracerebroventricular administration of the GABA<sub>B</sub> receptor agonist, baclofen, causes an increase in food intake in satiated pigs (69). Moreover, increased food intake obtained after administration of baclofen can be abolished by pretreatment with the GABA<sub>B</sub> receptor antagonist, phaclofen (69). In agreement, several evidences indicate that neurons in the hypothalamic arcuate nucleus express to a large extent the GABA transporter, VGAT (70, 71) as well as the GABA synthesizing enzymes, GAD65 and GAD67 (70). Using immunohistochemistry, GAD65/GAD67 and GABA immunoreactivities have been demonstrated in the majority of NPY/AgRP neurons located in the hypothalamic arcuate nucleus (70, 71). On the other hand, in spite of GAD65/GAD67 mRNA presence has been demonstrated in approximately one-third of POMC-expressing neurons (72), VGAT was not detected in hypothalamic POMC cell bodies (53) suggesting the absence of POMC GABA-releasing neurons.

To further understand the role of GABAergic neurons in appetite regulation, Tong et al. have shown that while both NPY and AgRP stimulate food intake when infused into the brain, the weight loss seen when AgRP-expressing cells are destroyed is recapitulated by targeted deletion of their ability to release GABA, rather than NPY or AgRP (73). Furthermore, the severe anorectic phenotype induced by the diphtheria-induced acute ablation of AgRP-expressing neurons in adult mice can be rescued with chronic infusion of a benzodiazepine, known to enhance GABA effect at the level of GABA<sub>A</sub> receptor (74). These evidences indicate that the synaptic release of GABA by AgRP-expressing neurons in the hypothalamic arcuate nucleus is required for normal regulation of energy balance. Wu et al. further explored the role of the GABAergic outputs of AgRP-expressing neurons. These authors found that in adult mice lacking AgRP-expressing neurons, pharmacological stimulation of GABA<sub>A</sub> receptors in the parabrachial nucleus, by means of local injection of bretazenil (a partial GABA<sub>A</sub> receptor agonist) is sufficient to maintain feeding. Wu and colleagues further corroborate these findings by examining the effects of either infusing a GABA antagonist directly into the parabrachial nucleus or selectively ablating AgRP inputs to this area. Both experiments induced a progressive decrease in food intake in mice, indicating that GABAergic inputs from arcuate nucleus AgRP-expressing neurons to the parabrachial nucleus are required to maintain a critical level of appetite stimulus (75). These observations clearly represent a potential shift away from early explanations of energy metabolism regulation, where GABA was thought to facilitate the feeding effect of NPY at target sites in the paraventricular nucleus by blocking opposing POMC transmission (76–78).

### GLUTAMATE AND GABA ACTIONS ON NEURONAL-ASTROCYTIC METABOLIC COUPLING MECHANISM UNDERLYING HYPOTHALAMIC APPETITE REGULATION

To date, glutamate and GABA actions on neuronal-astrocytic metabolic coupling mechanism underlying hypothalamic appetite regulation have been largely unexplored mainly due to the absence of appropriate *in vivo* methodological approaches. Earlier, a variety of *in vivo* Magnetic Resonance Imaging (MRI) and Magnetic Resonance Spectroscopy (MRS) methods have been shown to provide comprehensive information on cerebral activation and the underlying metabolic coupling mechanisms operating between neurons and astrocytes. However, the relatively large voxel size used in the acquisition of *in vivo* <sup>13</sup>C Magnetic Resonance spectra precludes its use for studying the relatively reduced appetite controlling hypothalamic area of small rodents. Alternatively, High-Resolution <sup>13</sup>C Nuclear Magnetic Resonance (NMR) spectroscopy investigations of the cerebral metabolism of tracers such as [1-<sup>13</sup>C]glucose or [2-<sup>13</sup>C]acetate contributed comprehensive information on the operation of the neuronal and astrocyte TCA cycles and the transcellular exchanges of glutamate–glutamine or GABA between neurons and astrocytes of the whole brain [see for example (79–82)].

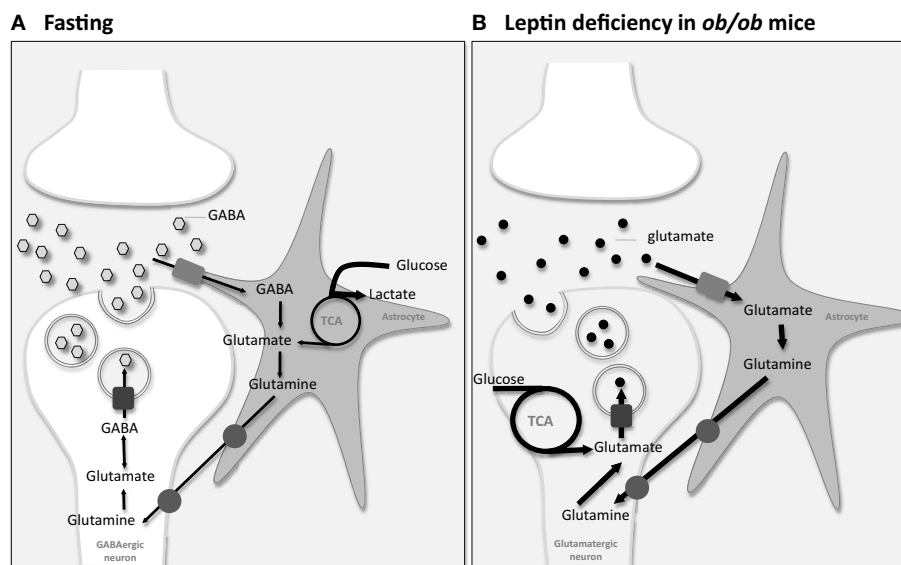
Nevertheless, the relatively large amounts of cerebral tissue needed to prepare brain extracts for high-resolution <sup>13</sup>C NMR spectroscopy constitutes an important limitation. To overcome the above-mentioned limitations, High-Resolution Magic Angle Spinning (HR-MAS) NMR spectroscopy, a technique yielding high quality spectra from very small tissue biopsies (5–10 mg, a size comparable to the size of the mice brain hypothalamus) was suggested to improve the spatial resolution and to investigate directly hypothalamic metabolism. Whereas <sup>1</sup>H HR-MAS NMR has been used for metabolic profiling of normal and diseased tissues (83), <sup>13</sup>C HR-MAS NMR spectroscopy offers the additional advantage of providing information on the operation of the metabolic pathways.

Recently, Violante et al. used <sup>13</sup>C HR-MAS NMR spectroscopy analysis of mice hypothalamic biopsies, after [1-<sup>13</sup>C]glucose injection, to better understand the mechanisms underlying neurotransmission events and the associated neuronal-astrocytic metabolic coupling mechanisms underlying hypothalamic appetite regulation (84). Following [1-<sup>13</sup>C]glucose injection, glycolytic and TCA cycle intermediates are labeled distinctively, providing information on the relative contribution of the corresponding metabolic pathways. Initially [1-<sup>13</sup>C]glucose is metabolized to [3-<sup>13</sup>C]pyruvate via glycolysis. Labeled pyruvate is then reduced to [3-<sup>13</sup>C]lactate by lactate dehydrogenase, or alternatively enters the TCA cycle, producing [4-<sup>13</sup>C]glutamate and [4-<sup>13</sup>C]glutamine. Moreover [4-<sup>13</sup>C]glutamate can be converted to [2-<sup>13</sup>C]GABA. On this basis, the authors have shown that appetite stimulation, during the feeding-fasting paradigm, increases significantly the <sup>13</sup>C incorporation in lactate carbons (84). Augmented lactate labeling most probably indicates a relatively increased glycolytic activity. Therefore, increased neuronal firing in the hypothalamus triggered by fasting may stimulate the use of lactate as neuronal fuel leading to increased astrocytic glucose consumption

and glycolysis. Moreover, fasted mice show increased hypothalamic  $[2-^{13}\text{C}]\text{GABA}$  content (84) most probably attributable to the existence of GABAergic neurons in key appetite regulation hypothalamic nuclei (70). Increased  $[2-^{13}\text{C}]\text{GABA}$  concentrations may be derived either from increased net synthesis or reduced net degradation. Potential increases in GABA synthesis involve an augmented activity of the glutamate-glutamine-GABA cycle, since glutamate and glutamine are considered the main precursors of GABA. Interestingly, despite the increase in the  $[2-^{13}\text{C}]\text{GABA}$  enrichment no increase was detected in  $[4-^{13}\text{C}]\text{glutamate}$  or  $[4-^{13}\text{C}]\text{glutamine}$ . Thus the increased  $[2-^{13}\text{C}]\text{GABA}$  concentration in the hypothalamus of fasted animals appears to result mainly from reduction in GABA metabolizing pathways, rather than increased GABA synthesis by augmented activity of the glutamate-glutamine-GABA cycle.

Using a similar methodology, we have recently studied the neuronal-astrocytic metabolic coupling mechanism underlying hypothalamic appetite stimulation in hyperphagic leptin-deficient *ob/ob* mice. After a meal, leptin is released from adipose tissue to bind to the hypothalamic leptin receptor inducing an anorexic response consisting of a reduction in food intake and an increase in energy expenditure. On the contrary, in fasting periods, decreased plasma levels of leptin promote increased food intake and diminished energy consumption (85). Disruptions in the leptin signaling systems are often associated with hyperphagia and consequently obesity. In the leptin-deficient *ob/ob*

mice, hypothalamic leptin signaling is drastically reduced and hyperphagia develops leading to obesity. We have showed that leptin deficiency in *ob/ob* mice resulted in significantly increased  $^{13}\text{C}$  incorporation from  $[1-^{13}\text{C}]\text{glucose}$  in glutamate and glutamine carbons of hypothalamic biopsies suggesting that leptin-dependent hypothalamic activation, contrary to fasting-induced appetite stimulation, involves mainly increases in neuronal oxidation and glutamatergic neurotransmission together with elevated glutamate-glutamine cycling (86). **Figure 2** provides an illustration on the use of  $^{13}\text{C}$  HR-MAS NMR spectroscopy to investigate appetite regulation in small hypothalamic areas during cerebral activation by different feeding activation paradigms. Unlike sensorial or motor paradigms [see for example (87–90)], where only glutamatergic or GABAergic terminals are involved in a simple activation/inhibition mechanisms, both glutamatergic and GABAergic stimulations on different neuronal populations may eventually lead to the dominant orexigenic or anorexigenic response, depending of their relative contributions. Most probably, the observed presence of both glutamatergic and GABAergic neurotransmission in association with different feeding activation paradigms may reflect the existence of complex feedback loops on the neuroendocrine regulation underlying appetite regulation. These feedback loops are crucial homeostatic mechanisms for the hypothalamic neuroendocrine regulation involving the operation of both peripheral signals and intrahypothalamic neurotransmitters.



**FIGURE 2 | Information on the integrated neuronal-astrocytic metabolic coupling mechanisms underlying appetite regulation can be investigated using  $[1-^{13}\text{C}]\text{glucose}$  injection followed by analysis by  $^{13}\text{C}$  High-Resolution Magic Angle Spinning (HR-MAS) Nuclear Magnetic Resonance (NMR) spectroscopy analysis of mice hypothalamus biopsies. (A) Fasting-induced changes: Violante et al. showed that increased neuronal firing in the hypothalamus triggered by fasting may stimulate the use of lactate as neuronal fuel leading to increased astrocytic glucose consumption and glycolysis (dark bold arrows). Moreover, fasted mice showed increased hypothalamic  $[2-^{13}\text{C}]\text{GABA}$  content most probably attributable to the existence of**

GABAergic neurons in the hypothalamus. Despite elevated  $[2-^{13}\text{C}]\text{GABA}$ , no increase was detected in the main precursors of GABA, glutamate, and glutamine, suggesting a reduction in GABA metabolizing pathways rather than increased GABA synthesis by augmented activity of the glutamate-glutamine-GABA cycle (84). **(B)** Leptin-deficiency-induced changes: we have shown that leptin deficiency in hyperphagic *ob/ob* mice resulted in significantly increased  $^{13}\text{C}$  incorporation from  $[1-^{13}\text{C}]\text{glucose}$  in glutamate and glutamine carbons of hypothalamic biopsies suggesting that leptin-dependent appetite activation involves mainly increases on neuronal oxidation and glutamatergic neurotransmission together with elevated glutamate-glutamine cycling (dark bold arrows) (86).



## CONCLUDING REMARKS

Although to date most effort has been placed on examining direct regulation of hypothalamic appetite by neuropeptide-expressing neurons, it is evident that hypothalamic neurons further release and respond to excitatory and inhibitory amino acid neurotransmitters, as glutamate and GABA. Neuropeptides and amino acids neurotransmitters may both function as independent transmitters, or alternatively, neuropeptides may work by modulating the actions of glutamate and GABA and vice-versa. Herein, current knowledge on neuronal-astrocytic interactions underlying glutamate- and GABA-dependent hypothalamic appetite stimulation was reviewed. Apparently, different feeding paradigms associated with appetite stimulation account for different responses in neuronal-astrocytic metabolic coupling mechanisms. Whereas the fasting state is associated both with the use of

lactate as neuronal fuel leading to increased astrocytic glucose consumption and with augmented hypothalamic GABAergic neurotransmission, elevated neuronal oxidation, and glutamatergic neurotransmission with increased glutamate-glutamine cycling are hallmarks of leptin deficiency in hyperphagic rodents. Further information on the neuronal-astrocytic metabolic pathways underlying appetite stimulation during different feeding paradigms, which can be achieved by the combined use of  $^{13}\text{C}$  HR-MAS NMR spectroscopy and metabolic tracers, may be a valuable tool for finding novel anti-obesity central-based therapeutical targets.

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