Physiology & Behavior 263 (2023) 114108

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh

Tanycyte, the neuron whisperer

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ARTICLE INFO

Keywords:
Arcuate nucleus
Tanycytes
Glia-neuron communication
Hypothalamus
Energy balance

ABSTRACT

Reciprocal communication between neurons and glia is essential for normal brain functioning and adequate physiological functions, including energy balance. In vertebrates, the homeostatic process that adjusts food intake and energy expenditure in line with physiological requirements is tightly controlled by numerous neural cell types located within the hypothalamus and the brainstem and organized in complex networks. Within these neural networks, peculiar ependymoglial cells called tanycytes are nowadays recognized as multifunctional players in the physiological mechanisms of appetite control, partly by modulating orexigenic and anorexigenic neurons. Here, we review recent advances in tanycytes' impact on hypothalamic neuronal activity, emphasizing on arcuate neurons.

1. Introduction

Glial cells are distinct neural cells involved in numerous physiological and physiopathological processes by supporting neuronal functions. Their diverse roles include, not exhaustively, the transport and sensing of nutrients, hormones, and neurotransmitters, the secretion of cytokines and gliotransmitters, and the energy supply for neuronal activity. Altogether, these glial functions allow the maintenance of numerous brain processes, such as synaptogenesis, synaptic transmission, information processing, and brain plasticity. The physiological functions affected by glial cells are, therefore, always linked to the output of neurons in their local network.

For two decades, numerous studies have shown that glial cells are integrated into feeding circuits [1–4] and regulate different processes that ensure the maintenance of energy balance, such as food intake, thermoregulation, metabolism, hormone secretion, hepatic glucose production, and glucose/fatty acid metabolism in peripheral tissues [5,6]. Among the diverse functions that allow these regulations, glial cells can transport nutrients into the brain, mainly glucose, to redistribute it to neurons as lactate [7]. Glia also display the capacity to sense a large range of metabolic humoral and hormonal information to transmit the information to neurons and modulate their functions. Alternatively, studies also showed that glial cells are susceptible to obesogenic diets, developing a reactive morphology that alters neuronal functions [3,8]. Thus, a coordinated and reciprocal dialog between glia and neurons is required for the system to work properly.

For the past decade, peculiar glial cells called tanycytes have attracted much attention for energy balance regulation [3,9–11]. Hypothalamic tanycytes are elongated ependymal cells composed of a cell body lining the walls and floor of the third ventricle [12,13] and a long basal process extending through the hypothalamic parenchyma contacting blood vessels and numerous neural cell types −including orexigenic and anorexigenic neurons controlling energy balance [14,15] (Fig. 1A-B). This unique polarized morphology allows them to form an integrative interface between different compartments: the ventricular system and the brain parenchyma on one side and the blood circulatory system on the other side. In addition, tanycytes present a diverse and dynamic gene expression profile allowing them to display distinct functional features related to metabolism [10]. Indeed, tanycytes first monitor brain-blood exchanges by controlling the secretion of neurohormones into the hypothalamus-pituitary portal circulation and by transporting nutrients and metabolic hormones, such as ghrelin, leptin, and insulin, into the brain [16–18]. Additionally, tanycytes sense and respond to changes in nutrient and hormonal levels, particularly via calcium signaling [19]. Altogether, tanycytes' peculiar morphology and distinct functional characteristics define them as "metabolic integrators" for regulating energy balance.

Following this integration, tanycytes would then convey the metabolic information to neurons. Indeed, others and we have shown that the detection of metabolic state by tanycytes in rats and mice is associated with changes in hypothalamic neuronal gene expression and activity, leading to physiological responses for the regulation of energy balance.

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https://doi.org/10.1016/j.physbeh.2023.114108
Received 17 December 2022; Received in revised form 23 January 2023; Accepted 1 February 2023
Available online 3 February 2023

However, the mechanisms underlying these processes remain poorly understood. This review will summarize what we know about tanycyte-neuron communication in controlling energy balance while illuminating possible avenues for future research.

2. Tanycytes modulate neuronal functions

Numerous studies using specific tanycyte ablation or alteration in tanycyte functions showed that tanycytes modulate hypothalamic neuronal functions, particularly those of neuropeptide Y/agouti related peptide (NPY/AgRP) and proopiomelanocortin/cocaine- and amphetamine-regulated transcript (POMC/CART) neurons. This neuronal modulation occurs via three main actions: by increasing or decreasing neuropeptide gene expression [20–24], by regulating neuronal cell signaling [16,25], and by altering neuronal firing [18,26,27].

2.1. Tanycytes regulate neuronal gene expression

Studies using tanycyte ablation were initially employed by different groups to evaluate the impact of tanycytes on neuronal functions and, subsequently, on mouse physiology. In this perspective, Sanders et al. reported that the destruction of tanycytes following an alloxan injection induced neuronal swelling and decreased the hypothalamic expression of *Npy* mRNA [20]. Two weeks after alloxan injection, the restoration of tanycytes was associated with a significant decrease in *Npy* and *Pomc* mRNA [20]. More recently, conditional median eminence (ME) and arcuate nucleus (ARH) tanycyte ablation showed that the expression of metabolic neuropeptides in hypothalamic regions in direct contact with tanycytes, such as *Npy, Cart, Gal, Sst, Trh, Oxt*, and *Avp,* was unaffected in basal conditions [28]. Such results were confirmed in our laboratory following the ablation of glucokinase-expressing tanycytes [29]. However, an alteration in gene expression was observed during energy imbalance. Indeed, the expected increase in *Agrp* and *Npy* mRNA induced by fasting was blunted following tanycyte ablation [29].

Other approaches using genetic manipulations to alter or modify

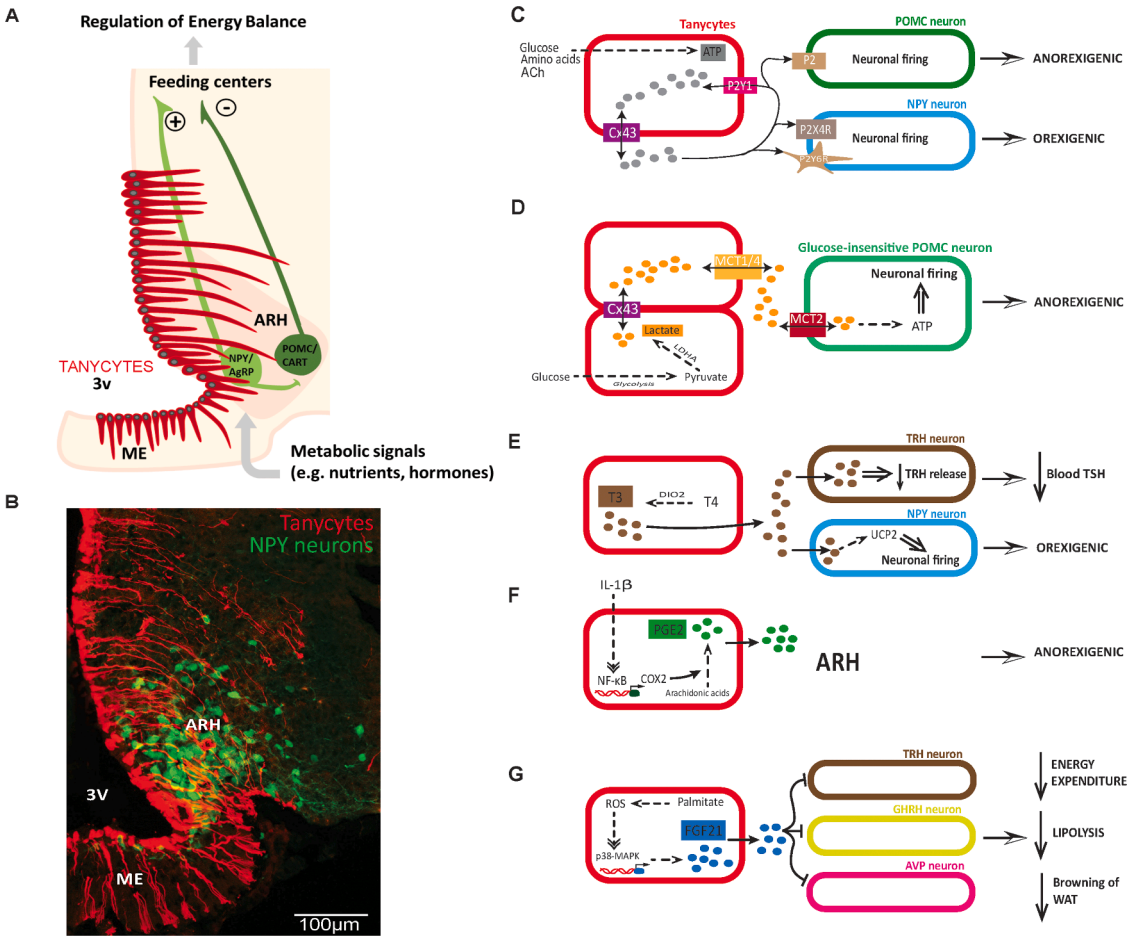

Fig. 1. Tanycyte signals for the regulation of energy balance. A. Schematic representation of tanycyte-neuron units. Tanycytes (in red) line the walls and floor of the third ventricle (3v) and send processes that make close contacts with NPY/AgRP neurons (light green) and POMC/CART neurons (dark green) located in the arcuate nucleus of the hypothalamus (ARH). **B.** Representative image of tdTomato-expressing tanycytes (in red) making close contact with GFP-expressing NPY neurons (in green) located in the ARH. **C.** ATP release by tanycytes. Glucose, amino acids, and acetylcholin are sensed by tanycytes and converted into ATP. ATP is then released by CX43 gap junctions and acts on tanycytes (red box), NPY (blue box), and POMC (green box) neurons. **D.** Lactate release by tanycytes. Glucose is metabolized in tanycytes into lactate which is transmitted through MCTs to glucose-insensitive POMC (green box) neurons, where it is converted to energy (ATP) to induce POMC neuronal firing. Lactate also travels throughout a tanycytic network through CX43 gap junctions to magnify metabolic signaling efficiency. **E.** T3 release by tanycytes. T4 is taken up by tanycytes from the circulation and converted into active T3 through type 2 deiodinase (DIO2). T3 is then released by tanycytes and modulates TRH neuron secretion and NPY neuron activity. **F.** PGE2 release by tanycytes. NEMO-dependent NF-κB in tanycytes is activated by systemic IL-1β, which induces the expression of *Cox2* and the release of the anorexigenic factor PGE2. **G.** FGF21 release by tanycytes. Palmitate oxidation in tanycytes triggers FGF21 expression via ROS/p38-MAPK pathway. FGF21 release by tanycytes reduces lipolysis, energy expenditure, and the browning of white adipose tissues (WAT). Scale bar: 100 µm in B. Dashed arrow: enzymatic reactions. Dashed double arrow: induction of transcription. Arrow: secretion.

tanycyte functions also revealed modulations of neuronal gene expression by tanycytes, suggesting that specific roles, such as their capacity to detect nutrients and hormones, also affect neuronal function [21–23]. For instance, deletion of *Glut2* (*i.e.*, glucose transporter) or *Mct1* (*i.e.*, monocarboxylate transporter) in tanycytes in rats resulted in the abolition of glucose-induced increase in *Pomc* and *Cart* mRNA and decrease in *Npy* and *Agrp* mRNA [21,22]. Similarly, *Glut2* deletion in mouse *Gfap*-expressing tanycytes induced a loss in refeeding-induced *Pomc* expression [30]. Alternatively, in fasting and refeeding conditions, mice lacking insulin receptors in tanycytes exhibit a significant increase in *Fos* expression in AgRP neurons but not in POMC neurons [18]. Finally, mice lacking leptin receptors in tanycytes display a significant increase in *Agrp* mRNA in basal conditions [31]. Altogether, these results highlight tanycytes' impact on the gene expression of distinct neuronal populations.

2.2. Tanycytes participate in the activation of neuronal signaling pathways

Diverse evidence also suggests that tanycytes modulate signaling pathways in hypothalamic nuclei. Tanycytes' ablation or functional alteration first modifies c-Fos activation following the fasting/refeeding paradigm. Indeed, ablation of glucokinase-expressing tanycytes decreased the number of c-Fos-positive cells in the ventromedial part of the ARH and increased it in the ventromedial nucleus (VMH) in response to fasting [29]. Conversely, refeeding-induced c-Fos activation in the dorsomedial ARH was blunted in mice with glucokinase-expressing tanycyte ablation [29]. In addition, *Glut2* deletion in mouse *Gfap*-expressing tanycytes also increased c-Fos in the VMH in response to fasting [30].

Response to hormones in the ARH may also be altered after tanycyte ablation or functional alteration, while not always reported and controversial. Indeed, regarding leptin sensitivity, ablation of *Gck*-expressing tanycytes resulted in an alteration in pSTAT3 activation in the ARH 45-minutes following leptin injection [29], whereas ablation of ME and ARH tanycytes does not 15-minutes following leptin injection [28]. Similarly, knockout of leptin receptors in tanycytes has been shown to diminish pSTAT3 activation by about 30% in the ARH compared to control littermates [31], whereas another study did not observe such an effect [32]. Similarly, deletion of insulin receptors in tanycytes induced no notable differences in cellular leptin sensitivity compared to control mice, whereas it resulted in a noteworthy reduction of pAKT immunoreactive cells in the ARH in response to insulin [18].

2.3. Tanycytes modulate neuronal calcium dynamics and membrane potential

Recent studies revealed that tanycytes can modulate neuronal electrical activity besides gene expression and signaling pathways. *Ex vivo* activation of calcium-permeable channelrhodopsin specifically expressed by tanycytes leads to depolarized orexigenic (NPY/AgRP) and anorexigenic (POMC) neurons via an ATP-dependent mechanism [26]. Alternatively, Lhomme et al. showed that tanycytes can drive POMC activity via lactate metabolism [27]. Finally, AgRP neuronal activity measured by calcium dynamics is altered following tanycyte-specific insulin receptor deletion [18]. Indeed, response to food presentation in fasted animals, characterized by a rapid inhibition of AgRP neurons, was abolished, whereas ghrelin injection in fed animals, inducing a quick activation of AgRP neurons, was largely attenuated in knockout mice [18]. Thus, tanycytes can regulate different aspects of neuronal functions in diverse populations.

3. What about tanycyte signals?

While neuronal modulation by tanycytes can occur in indirect ways, by controlling the access of nutrients and hormones to hypothalamic neurons for instance [16,17,33,34], tanycytes are also able to regulate neuronal functions by releasing both orexigenic and anorexigenic molecules (Fig. 1C-G), what we previously called "tanykines" [35].

3.1. Tanycyte purines regulate food intake and energy expenditure

Several studies showed that tanycytes release ATP following nutrient sensing (Fig. 1C) [19,26,36,37]. ATP is an extracellular purine that mediates signaling inside and between cells. In response to various physiological and pathological stimuli, cells can release ATP via different ways, including exocytosis and large conductance channels, such as hemichannels, pannexin channels, volume-regulated anion channels, and P2X7 receptors [38]. Furthermore, released ATP is rapidly converted into adenosine by ectonucleotidases. Extracellular levels of adenosine are then controlled by glial cells, especially by astrocytes, through adenosine kinase (ADK) and nucleoside membrane transporters [38]. ATP and its metabolite then act through purinergic receptors, classified as adenosine P1Rs and ATP/ADP P2Rs (*i.e.*, P2XRs and P2YRs). Expressed in various neural cell types, these receptors, once activated, induce an increase in intracellular calcium (Ca^{2+}) [39].

In the hypothalamus, *in vivo* induction of tanycytic ATP release by optostimulation induces the depolarization of POMC and NPY neurons, whereas P2 receptor antagonists completely abolish this depolarization [26]. These depolarizations finally lead to acute hyperphagia, demonstrating that the balance between NPY and POMC neurons activation shifts toward the orexigenic pathway in these experimental conditions [26]. Indeed, both NPY/AgRP neurons and POMC neurons [40–42], as well as glial cells, abundantly express purinergic receptors in the hypothalamus. However, functional presynaptic P2X4 receptors in terminals of NPY/AgRP, where their activation by ATP facilitates GABA release on POMC neurons and paraventricular nucleus neurons [42]. Therefore, tanycytic ATP may mediate inhibitory presynaptic inputs on POMC neurons.

In addition, tanycytes express ecto-ATPase NTDDase2, which converts ATP into adenosine (Fig. 1C), which has been shown to mediate NPY/AgRP neuron inactivation through adenosine A1 receptors [43,44]. Therefore, tanycyte action via purines remains ambivalent and more studies are still needed to determine how these tanycytic signals are decoded by the different ARH neuronal populations for intercellular communication.

Interestingly, recent evidence showed the involvement of the purinergic system in the pathogenesis of obesity and the potential therapeutic use of purinergic compounds to treat it [45]. In obesity, hypothalamic UDP concentrations are elevated due to an increased circulating source of uridine: this elevation participates in the overstimulation of feeding via the P2Y6-dependent activation of AgRP neurons [45]. Additionally, the development of selective antagonists for purinergic receptors has corroborated the evidence that pharmacological inhibition of P2Y6R signaling in AgRP neurons reduces food intake and improves systemic insulin sensitivity in obese mice [46].

Taken together, these results showed that purines play critical roles in mediating the communication between tanycytes and neurons following the tanycytic detection of glucose and amino acids in plasma and CSF after a meal. These purines act on tanycytes and neurons by affecting their excitability and metabolism.

3.2. Tanycyte lactate fuels neurons regulating energy balance

It is now admitted that astrocytes sense circulating glucose and participate in glucose uptake to meet the high energy demands of neighboring neurons [7]. Glucose uptake by astrocytes is proportional to astrocytic glutamate uptake, which in turn depends on the glutamatergic activity at the synapse. Glucose taken up by astrocytes enters the glycolytic pathway to generate lactate, which is then transferred to neurons through monocarboxylate transporters (MCTs). In neurons, it is finally converted into pyruvate for aerobic energy production (*i.e.*, ATP)

in the mitochondria [7]. MCT1 and MCT4 are specifically expressed in glial cells and serve as lactate importers or exporters depending on the metabolic state, whereas MCT2 is mainly expressed by neurons and mediates lactate entry into neurons [7].

As glial cells, tanycytes also convert glucose into lactate and transport it to neighboring neurons through MCT1 and MCT4, thereby stimulating ATP synthesis necessary for neuronal function (Fig. 1D) [22]. Notably, it has been shown that the tanycyte network mediated by the gap junction protein connexin 43 (CX43) shuttles lactate between tanycytes. This lactate is then transferred to glucose-insensitive POMC neurons to fuel this neuronal population (Fig. 1D). Indeed, selective suppression of either tanycytic MCTs or CX43 resulted in altered POMC neuronal activity, feeding behavior, and energy metabolism in mice [27] (Fig. 1D). Although it is not clear whether tanycytes form a tandem with a specific subpopulation of glucose-insensitive POMC neurons, these results indicate that tanycytes not only shuttle but also actively process peripheral information (*e.g.*, glucose to lactate translocation) and transmit it to the neuronal circuitry, depending on fluctuating systemic glucose levels [27].

3.3. The balance between deiodinase2/deiodinase3 in tanycytes regulates feeding

Another case of tanycyte-processed peripheral information is the regulation of triiodothyronine (T3) levels in the hypothalamus [47]. Indeed, T3 levels are regulated by the balance between deiodinase2 and deiodinase3 enzymes expressed by tanycytes (Fig. 1E) and then regulate a large panel of genes and functions [48]. First, hypothalamic T3 controls mitochondrial proliferation in NPY/AgRP neurons, increasing their excitability and inducing meal initiation in mice [47] (Fig. 1E). Another possible route of action for tanycytic T3 in energy balance could be via the thyrotropin-releasing hormone (TRH) neurons located in the paraventricular nucleus (PVN) [49]. These neurons project towards the median eminence, where tanycyte endfeet surround their axonal terminals to control the release of TRH into the pituitary-portal circulation. TRH stimulates the secretion of thyroid-stimulating hormone (TSH), which then acts on the thyroid gland to produce L-thyroxine (T4) [49, 50] (Fig. 1E). Tanycytes uptake T4 from the circulation and convert it into active T3, resulting in negative feedback on neural TRH production. Finally, tanycyte-derived T3 can act on tanycytes themselves. Indeed, T3 administration increases the expression of retinaldehyde dehydrogenase 1 enzyme (RALDH1) in tanycytes: this enzyme converts retinaldehyde into retinoic acid, which is known to increase *Agrp* and *Pomc* gene expression in *ex vivo* hypothalamic rat explants [51].

3.4. Tanycyte endozepines and feeding-related behavior

Glial cells express polypeptide diazepam-binding inhibitor (DBI), which generates several regulatory peptides via proteolytic cleavage, including the anorexigenic octadecaneuropeptide (ODN) [36]. DBI is highly expressed in glial cells in the hypothalamus, especially in tanycytes, and was proposed as a paracrine factor with a positive effect on diet-induced obesity [36]. Indeed, the intracerebroventricular injection of ODN in rats provokes a reduction of *Npy* mRNA and an increase of *Pomc* mRNA levels, suggesting that the anorexigenic action of ODN is mediated via inhibition of NPY neurons and/or activation of POMC neurons. In another study, Lanfray et al. also revealed that DBI expressed by some POMC and GABAergic neurons in the ARH is processed into the bioactive peptide nonadecaneuropeptide (NDN). NDN production within the ARH is modulated by fasting and refeeding. Similarly to ODN, NDN exerts a potent anorexigenic action which is relayed by the activation of the melanocortin receptor type 4 (MC4R) [52].

Mechanistically, glial DBI can induce STAT3 phosphorylation in hypothalamic neurons in wild-type mice but not in leptin-deficient mice (ob/ob mice) or in mice where LepR is selectively knocked out in tanycytes, suggesting that endozepines mediate their anti-obesity effects by activating tanycytic leptin shuttles [53]. Indeed, while some authors did not detect LepR transcript in tanycytes [32], partly due to the limited detection of low-expressed genes using RNA sequencing, recent evidence showed that LepR signaling allows the transport of leptin through the tanycyte endosomal system [16,31].

3.5. Tanycytes signal via anti- and proinflammatory cues

Brain inflammation promotes the onset of obesity and diabetes. High-fat diet (HFD) feeding can cause neuronal leptin and insulin resistance to promote positive energy balance [8]. This resistance has been linked to the activation of inflammatory signaling cascades observed in central and peripheral metabolic tissues, such as the liver, skeletal muscle, and adipose tissue [54–56]. Within only three hours [57] or three days [58] of HFD feeding, the expression of proinflammatory cytokines increases in the hypothalamus. These acute changes correlate with various cellular responses, including gliosis and an alteration in brain vasculature and blood-brain barrier integrity [58–61]. Few studies suggested a possible role for tanycytes in this context by sensing and releasing pro- and anti-inflammatory cues. Indeed, in addition to metabolic hormones and regulators, tanycytes first act as gatekeepers for peripheral immune signals and mediate the central effects of systemic inflammation in mice. Systemic inflammation is marked by a notable increase and release of proinflammatory cytokines such as IL-1β. IL-1 β then activates NF-kB in VMH and dorsomedial ARH tanycytes, which in turn induces the expression of Cox-2 and the release of anorexigenic prostaglandins from tanycytes [62] (Fig. 1F).

Geller et al. showed that tanycytes also secrete fibroblast growth factor 21 (FGF21) [63], which is known to modulate neuroinflammation and oxidative stress by enhancing mitochondrial functions through AMPKa/AKT and by inhibiting the NF-kB pathway [64]. FGF21 deletion in tanycytes alters their lipid sensing, increasing fatty acid oxidation in white adipose tissues: this lipid mobilization and browning results in an increase in energy expenditure and a decrease in fat accumulation [63]. At the neuronal level, *Fgf21* deletion in tanycytes increases *Avp, Ghrh*, and *Trh* expression in the hypothalamus. These neuropeptides regulate cellular metabolism, energy expenditure, and lipid utilization through hypothalamic-pituitary axes and the sympathetic nervous system (Fig. 1G) [63]. This tanycyte-to-neuron communication raises the question of how tanycyte signals could reach parts of the brain that are anatomically distant. One possible way would be volume transmission, a mode of intercellular communication through the CSF [65]. Nevertheless, in contrast to this first report [63], Zhou et al. did not detect *Fgf21* expression in tanycytes using a mouse model that drives CRE recombinase in cells expressing FGF21 and deposited RNA sequencing datasets [66]. According to this study, FGF21 immunoreactivity in tanycytes might come from the bloodstream. Indeed, Pena-Leon et al. recently demonstrated that tanycytes control the hepatic FGF21 access into the rat hypothalamus [67]. However, this previous study also found *Fgf21* transcript expression in tanycytes [67], suggesting that at least part of the FGF21 immunoreactivity visualized in tanycytes arises from a local synthesis [68].

Finally, tanycytes also express chemerin, which is elevated in obesity and associated with insulin resistance [69]. Chemerin is an anti-inflammatory chemokine with autocrine, paracrine, and even endocrine roles *in vivo* [70]. Its positive effect on neuroinflammation occurs via the activation of calcium/calmodulin-dependent protein kinase kinase 2 (CAMKK2), adenosine monophosphate-activated protein kinase (AMPK), and nuclear factor erythroid 2-related factor 2 (Mrf2) [71]. In the hypothalamus, chemerin is highly expressed in tanycytes, controlled by the βTSH signal, and acts on ependymocytes, neurons, and tanycytes themselves [72]. Interestingly, in seasonal animals, chemerin has both anorexigenic and orexigenic effects according to the photoperiod. Indeed, intracerebroventricular injections of chemerin in F344 rats during a short photoperiod increase food intake. In contrast, similar injections during a long photoperiod decrease food intake, inducing

bodyweight reduction [72]. These results support the hypothesis that tanycytes can modulate both orexigenic and anorexigenic pathways via inflammatory cues.

4. Cell biology underlying tanycyte-neuron communication

4.1. Tanycyte morphology

Tanycyte-neuron communication likely relies on tanycyte morphology. Indeed, unlike typical ependymal cells, tanycytes extend long basal processes into the brain parenchyma, making numerous contacts with different neuronal populations, such as NPY, POMC, TH-containing, and KNDy neurons [15]. These contacts are established with neuronal soma as well as glutamatergic and GABAergic synapses [15]. Additionally, the tanycyte ultrastructure provides several clues regarding the molecular mechanisms that could underly tanycyte-neuron communication. Indeed, different organelles, including the endoplasmic reticulum (ER), mitochondria, vesicles, and cytoskeleton [15], suggest the importance of Ca^{2+} signaling and the vesicular system (Fig. 2A-C).

4.2. Tanycyte Ca^{2+} homeostasis for tanycyte-neuron communication

Like other glial cells, tanycytes act via changes in intracellular Ca^{2+}. Regarding energy balance regulation, studies have highlighted that Ca^{2+} increase can be induced in tanycytes by diverse nutrients and hormones, such as leptin [31], glucose [19], histamine [19], acetylcholine [19], and amino acids like arginine, lysine, and alanine [37]. However, the mechanisms underlying the glucose-induced Ca^{2+} increase remain the most described. Using acute brain slices incubated in artificial cerebrospinal fluid (aCSF), Frayling and colleagues showed that, although the application of aCSF containing 3 and 5 mM of glucose barely evoked Ca^{2+} responses in α-tanycytes, puffing glucose directly into them considerably increased the tanycytic Ca^{2+} response [19]. Therefore, tanycytes could preferentially detect through their cell bodies glucose in the CSF, where its concentration is likely more variable compared to the rest of the brain parenchyma [19]. Importantly, glucose also increases the intracellular Ca^{2+} signal of cultured β-tanycytes in a concentration-dependent manner [73].

The increase in tanycyte intracellular Ca^{2+} signal in response to glucose can occur via diverse mechanisms involving glucose sensing

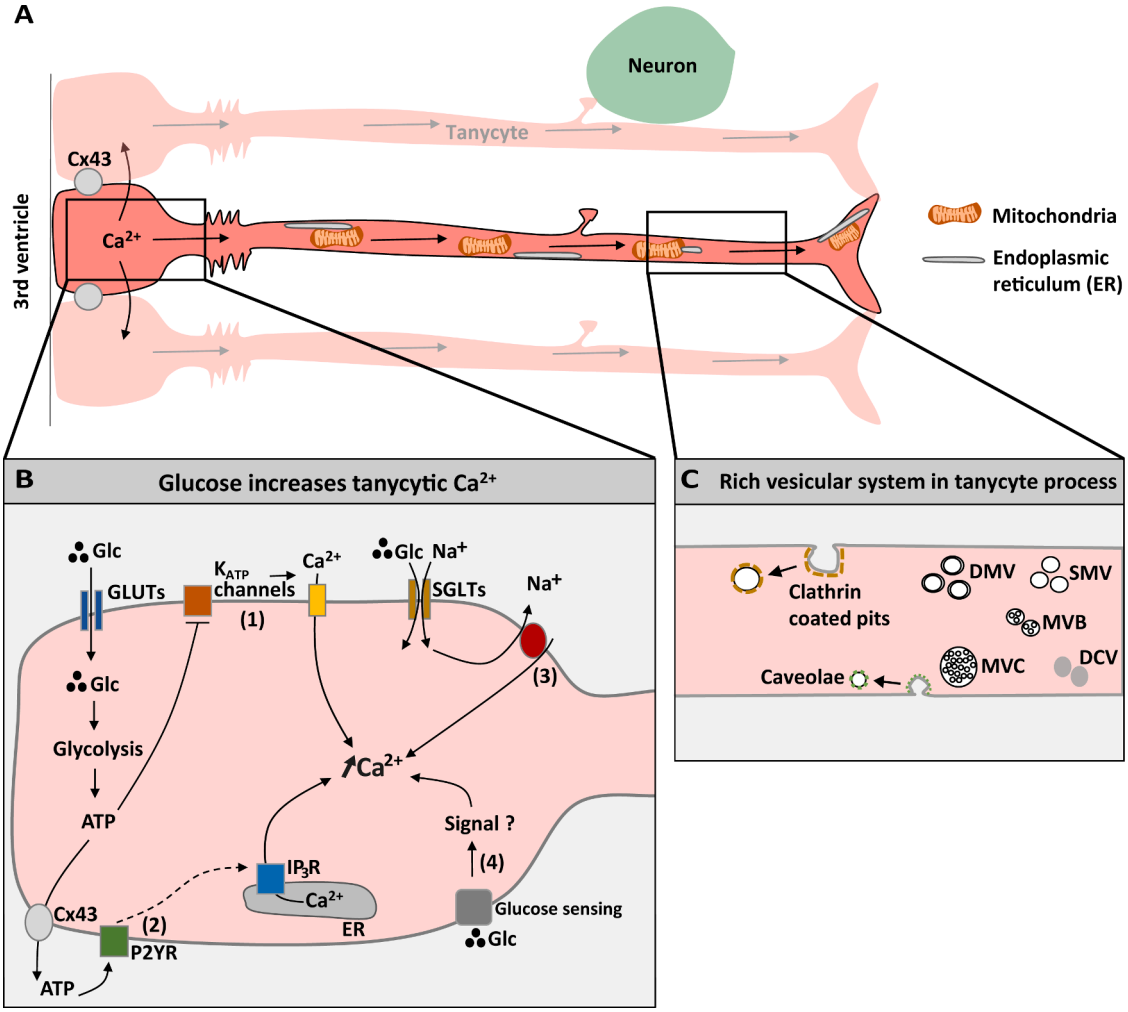

Fig. 2. Cell biology underlying tanycyte-neuron communication. A. Tanycyte Ca^{2+} waves are necessary for tanycyte-neuron communication. An increase in tanycyte intracellular Ca^{2+} can produce a Ca^{2+} wave that spreads both throughout tanycyte processes and to neighboring tanycytes, dorsally and ventrally. Cellular Ca^{2+} stores like the endoplasmic reticulum and mitochondria are present from the tanycyte cell body to the endfoot. **B.** Glucose increases intracellular Ca^{2+} in tanycytes by increasing ATP, which (1) closes ATP-sensitive *K*+ channels, inducing Ca^{2+} entry, and (2) is released through connexin 43 hemichannels (Cx43) and stimulates P2Y receptors (P2YR), subsequently mobilizing intracellular Ca^{2+} stores; (3) generating a Na^{+} entry through sodium-glucose cotransporters (SGLTs), which induces Ca^{2+} entry through the Na^{+}/Ca^{2+} exchanger; and (4) by a mechanism of glucose-sensing by receptors (*e.g.*, taste receptor). **C.** Tanycytes contain a rich machinery for endocytosis and exocytosis, including clathrin- and caveolin-coated pits, as well as single membrane vesicles (SMV), double-membrane vesicles (DMV), multivesicular bodies (MVB), dense core vesicles (DCV), and multivesicular cargoes (MVC).

and/or transport into the cell (Fig. 2B). First, tanycytes express well-known glucose-sensing proteins such as GLUT2, glucokinase, and ATP-sensitive K^+ channels. Thus, glucose can be transported into the cell through glucose transporters such as GLUT2 [74] and, to a lesser extent, CX43 hemichannels [75]. After that, glucose is metabolized within the cell through glycolysis, increasing the cellular ATP/ADP ratio: the increase in ATP closes ATP-sensitive K^+ channels, depolarizes the plasma membrane, and induces Ca^{2+} entry [76]. However, non-metabolizable glucose analogs also increase the intra-tanycyte Ca^{2+} signal, suggesting that glucose could cause intracellular tanycyte Ca^{2+} rise by other glucose-sensing mechanisms [19]. Indeed, glucose can also bind to a G protein-coupled receptor (*e.g.*, taste receptors and metabotropic glutamate receptors) to trigger Ca^{2+} mobilization [37]. Alternatively, glucose can be transported through the Na^+-dependent glucose cotransporters (SGLTs), generating a cellular Na^+ entry. This Na^+ influx subsequently induces Ca^{2+} entry through the Na^+/Ca^{2+} exchanger, thus increasing intracellular Ca^{2+} [77]. While these mechanisms described the extracellular space as the source of Ca^{2+}, intracellular stores may also be mobilized. Indeed, the rise in Ca^{2+} signal observed in cultured β-tanycytes seemed to originate from the release of intracellular Ca^{2+} stores and not from the extracellular space via the opening of Ca^{2+} channels [73]. Accordingly, the increase in ATP induced by glucose can lead to the release of ATP through CX43. This ATP then stimulates P2Y receptors and subsequently mobilizes intracellular Ca^{2+} stores [26,73]. Notably, the endoplasmic reticulum (ER), the major cellular Ca^{2+} store, and mitochondria, another Ca^{2+} storing organelle, may play crucial roles in Ca^{2+} mobilization. Electron microscopy revealed the presence of such organelles from tanycyte cell bodies up to tanycyte endfeet [15]. To summarize, glucose may rise intracellular Ca^{2+} levels both by increasing Ca^{2+} entry from the extracellular space and releasing intracellular Ca^{2+} stores.

Intracellular Ca^{2+} can then spread along tanycyte processes via Ca^{2+} waves (Fig. 2A). Indeed, by activating tanycytes via the expression of Ca^{2+}-permeable channelrhodopsin, Bolborea and colleagues showed that a Ca^{2+} wave initiated within tanycyte cell bodies spreads along their processes at around 2.4 μm/s [26]. Thus, the activation of one tanycyte could reach, via its long process, neurons located farther away in the parenchyma. Indeed, the increase in tanycyte intracellular Ca^{2+} can induce the depolarization of both orexigenic NPY/AgRP and anorexigenic POMC/CART neurons followed by hyperphagia in mice [26], suggesting that tanycyte Ca^{2+} waves mediate tanycyte-neuron communication and have an effect on energy balance.

4.3. Tanycyte networks for tanycyte-neuron communication

Activating a single tanycyte also increases the intracellular Ca^{2+} signal in neighboring tanycytes, both dorsally and ventrally [26] (Fig. 2A), suggesting that tanycytes form a functional network. This inter-tanycyte spreading Ca^{2+} wave traveled at around 1 μm/s [26]. Studies showed that α- and β-tanycytes form physical networks between them [27,78] and with few parenchymal cells, including astrocytes and oligodendrocytes [78], mainly via CX43 gap junctions [27,78]. Indeed, *Cx43* mRNA was 1300 times higher than *Cx26* and 2000 times higher than *Cx30* in tanycyte cultures [78]. Moreover, by filling a single tanycyte with biocytin, a connexin-permeable molecule, two studies showed that the gap-junction diffusing molecule spreads to neighboring tanycytes [27,78]. Interestingly, an increase in the activity of connexin hemichannels (specifically, CX32) has also been shown to be preceded by an increase in intracellular Ca^{2+} concentration in Hela cells [79], raising the possibility that an inter-tanycyte spreading Ca^{2+} wave may sustain tanycyte connexin hemichannel activity. Notably, the loss of gap junctions via a non-specific gap junction blocker, carbenoxolone, is enough to decrease 2.3-fold transmembrane currents in α-tanycytes, suggesting that tanycyte networks are necessary to maintain overall tanycyte activity [78]. Furthermore, the tanycyte-induced POMC neuron firing activity is decreased in the presence of the gap-junction blocker carbenoxolone or the knocking-out of *Cx43* in tanycytes, indicating that physical and functional tanycyte networks are necessary for the neuronal activity [27].

4.4. Communication through the vesicular system

As shown in other cell types, Ca^{2+} mediates protein synthesis initiation [80,81], cytoskeleton reorganization [82], and the release of extracellular vesicles (EVs) [83,84]. Interestingly, tanycytes contain a rich machinery for endocytosis and exocytosis, consistent with their role in the transport of molecules [16,17,31] (Fig. 2C). Indeed, fluorescence and electron microscopy data revealed that tanycytes contain different types of molecules, such as clathrin or caveolin, or vesicles, such as single-membrane vesicles, double-membrane vesicles, multivesicular bodies, multivesicular cargoes, and dense-core vesicles [12,15,85], suggesting cell-to-cell communications (Fig. 2C).

Endocytosis occurs through different ways in tanycytes. Clathrin-mediated endocytosis is a vesicular transport that participates in the internalization of nutrients, receptors involved in signal transduction, and synaptic vesicle retrieval and reformation [86]. Clathrin has also been shown to participate in the exchange between synaptic boutons [87]. β1- and β2- tanycytes express clathrin at the ventricular cell pole [88]. Moreover, immunofluorescence data on α-tanycytes, which modulate neuronal function [26], highlighted the presence of clathrin in tanycyte boutons [15]. These data suggest that clathrin-mediated endocytosis could occur in different regions and subcellular parts of tanycytes, including those in contact with hypothalamic neurons [15]. Few molecules, such as leptin [16] and ghrelin [17], are already described to be internalized through clathrin-coated vesicles in tanycyte cultures.

Alternatively, caveolae are also involved in endocytosis and transcytosis. Caveolae are membrane microdomains that contain caveolin and have been shown to regulate intercellular communication close to gap junctions [89]. β1- and β2- tanycytes express caveolin-1 at the ventricular cell pole and their endfeet [88]. Additionally, the presence of gap junctions at these subcellular parts of tanycytes [12] raises the possibility that caveolae-mediated communication could occur at gap junctions between tanycytes and their partners.

Besides endocytosis, tanycytes could also communicate with neurons by releasing EVs. EVs are lipid-bound vesicles secreted by cells into the extracellular space and mediate glial cell-to-neuron communication [90,91]. Different types of EVs, including microvesicles and exosomes, differ in their biogenesis, release pathways, content or cargo, and function [92]. Importantly, tanycytes express CD9, a marker of exosomal formation and secretion [93], in their endfeet [15], suggesting that exosomes could be released from a tanycyte subcellular location in close contact with neurons. Müller glia, which are remaining radial glial cells as tanycytes [10,94], also express and release EVs labeled for CD9 and CD31 (for exosome maturation and target cell binding) [93], supporting the hypothesis of an EV-based communication mechanism in these glial cells [95]. Furthermore, a study by Zhang and colleagues suggests that hypothalamic stem cells along the third ventricle (*i.e.*, tanycytes) also express CD81-labeled exosomes and control aging speed via exosomal miRNAs [96]. However, in the latter study, the cell cultures were performed from the hypothalamus and the hippocampus, which include other stem cells besides tanycytes [96]. Additionally, electron microscopy data suggest that tanycytes contain dense-core vesicles [15]. Such vesicles are already described in neurons and glial cells and are known to contain neurotransmitters, proteins, and microRNAs [97–99]. Considered neurosecretory vesicles [100–102], these dense-core vesicles may represent another means of communication for tanycytes.

Finally, vesicle exocytosis by tanycytes is likely mediated by Soluble N-ethylmaleimide-sensitive-factor Attachment protein Receptor (SNARE). Indeed, liraglutide, an anti-diabetic and anti-obesity drug, has been recently shown to be taken up and secreted by tanycytes through transcytosis [103]. The authors showed that inhibiting tanycyte

SNARE-mediated exocytosis blunts the liraglutide-mediated neuronal activation and associated anti-obesity effect [103]. These results highlight the importance of exocytosis for tanycyte-neuron communication and the associated regulation of energy balance.

5. Conclusion

Within the hypothalamus, multiple cell types, including tanycytes, neurons, astrocytes, ependymal cells, endothelial cells, microglia, and oligodendrocytes, work together to respond and adapt to metabolic changes. Tanycytes interact through different signals with neurons to modulate their activity. Understanding the tanycyte regulatory network and its plasticity according to changes in energy balance will be essential to explain the regulation of energy balance and develop new therapeutic strategies for obesity and metabolic syndrome.

Perspectives and significance

Tanycytes are involved in the regulation of energy balance and glucose homeostasis via interactions with key hypothalamic neuronal populations, in particular NPY and POMC neurons. While the molecular mechanisms underlying these interactions are yet to be understood, their physiological consequences are multiple and may generate a negative or positive balance, prompting us to investigate how tanycytes modulate neuronal functions.

Acknowledgement

This work is supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (No. 948196), the Swiss National Science Foundation (PCEFP3_194551), Novartis Foundation for medical-biological research, The European Foundation for the Study of Diabetes, the Department of Biomedical Sciences, and the Faculty of Biology and Medicine at University of Lausanne.

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