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Blood-to-brain communication in the hypothalamus for energy intake regulation

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ABSTRACT

The arcuate nucleus (Arc) integrates circulating hormonal and metabolic signals to control energy expenditure and intake. One of the most important routes that enables the Arc to sense circulating molecules is through the median eminence (ME), which lacks a typical blood-brain barrier. However, the mechanism by which circulating molecules reach the Arc neurons remains unclear. This review focuses on what is known to date regarding the special structure and permeability of the ME vasculature and active transport of circulating molecules from the ME to the Arc. Recent studies have demonstrated that the ME displays angiogenic behavior that is expected to provide high vascular permeability. Parenchymal diffusion of circulating molecules from the ME vasculature is size-dependent, and tanycytes actively transport circulating molecules from the ME to the Arc. Finally, we highlight structural plasticity of the Arc and ME as playing an important role in maintaining energy balance homeostasis.

1. Introduction

The vascular system makes essential contributions to bidirectional brain-body communication. Of particular importance, to properly regulate energy balance, the brain must acquire information from circulating molecules that indicate the body's energy status. In this context, the hypothalamus is a key organ in the regulation of food intake. Early studies demonstrated that experimentally produced lesions of the hypothalamus generally result in obesity (Elmquist et al., 1999; Olney, 1969). The arcuate nucleus (Arc), located at the base of the mediobasal hypothalamus, contains neurons that sense circulating hormones and nutrients, which represent the body's energy needs, and respond to these signals by regulating feeding behavior to maintain energy homeostasis (Dietrich and Horvath, 2013; Elmquist et al., 1999). The Arc contains two antagonistic neuronal populations: the orexigenic NPY (neuropeptide Y)/AgRP (agouti-related peptide) neurons and the anorexigenic POMC (pro-opiomelanocortin)/CART (cocaine- and amphetamine-regulated transcript) neurons (for a review, see Sobrino Crespo et al., 2014). Circulating metabolic hormones, including leptin, insulin and ghrelin, and nutrients such as glucose, free fatty acids and amino acids must rapidly access the parenchyma of the Arc to activate or suppress these neurons. However, the brain protects its own homeostasis by carefully controlling what can and cannot enter from blood using the blood-brain barrier (BBB) at the capillary wall (Daneman et al., 2010; Engelhardt, 2003) and the blood-cerebrospinal fluid barrier (blood-CSF barrier) in the choroid plexus (for a review, see Ghersi-Egea et al., 2018). How circulating hormones and nutrients can reach Arc neurons is a matter of debate, but several routes have been proposed, and can be divided into two major groups: first, saturable receptor-mediated transport at the level of the BBB and blood-CSF barrier; and second, through the permeable vasculature in the median eminence (ME), which is adjacent to the Arc. The ME acts as a gateway for secretion of hypothalamic releasing hormones, and is a so-called secretory circumventricular organ. Since the ME lacks a typical BBB, it is also considered a gateway for circulating metabolic hormones and nutrients to access the Arc. This review focuses on the structural basis and role of the ME as a key area for blood-to-Arc communication.

2. Blood-brain barrier and blood-cerebrospinal fluid barrier

The BBB separates the brain parenchyma from blood (Zlokovic, 2008). The first checkpoint for circulating molecules to cross the BBB into the brain is the endothelial cells (Fig. 1). Tight junctions between endothelial cells are responsible for preventing passive paracellular

Abbreviations: Arc, arcuate nucleus; AgRP, agouti-related peptide; BBB, blood-brain barrier; BSA, bovine serum albumin; CART, cocaine- and amphetamine-regulated transcript; CSF, cerebrospinal fluid; FITC, fluorescein isothiocyanate; ME, median eminence; MW, molecular weight; NPY, neuropeptide Y; PDGFRβ, platelet-derived growth factor receptor β; POMC, pro-opiomelanocortin; VEGF, vascular endothelial growth factor

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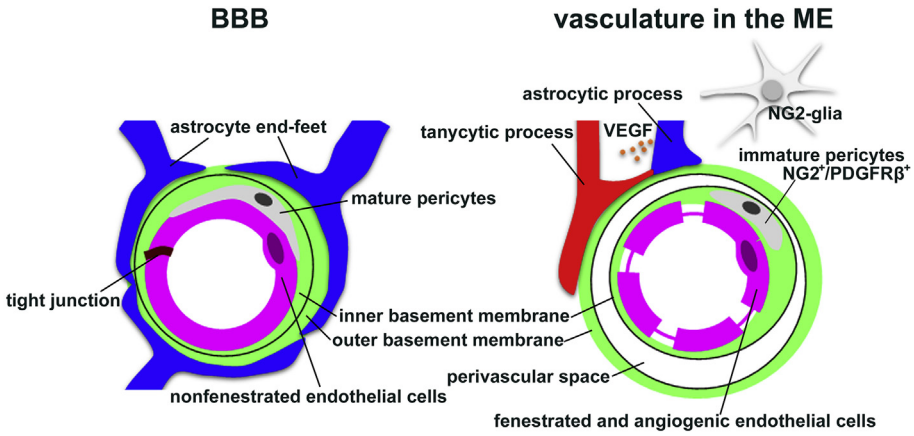

Fig. 1. Schematic representations of the BBB and vasculature in the ME.

diffusion of circulating molecules into the brain parenchyma (Reese and Karnovsky, 1967). Thus, brain capillary endothelial cells and tight junction components broadly determine BBB permeability (Braniste et al., 2014; for a review, see Jia et al., 2014; Ouyang et al., 2014). Astrocytes, which are involved in BBB integrity, encircle and interact with endothelial cells through their end-feet. Astrocytes release several molecules that influence BBB function. For example, vascular endothelial growth factor (VEGF) and matrix metalloproteinases down-regulate tight junction components and degrade the extracellular matrix (Annese et al., 2015; Argaw et al., 2009; for a review, see Cabezas et al., 2014). Conversely, angiopoietin-1 decreases BBB permeability by up-regulating tight junction components (for a review, see Alvarez et al., 2013). Pericytes are involved in the establishment and maintenance of the BBB (Armulik et al., 2010; Bell et al., 2010; Daneman et al., 2010). Pericyte deficiency down-regulates tight junction components and increases BBB permeability (Armulik et al., 2010). Basement membranes between the endothelial cells and the astrocytic end-feet are also involved in BBB functions (Menezes et al., 2014). Two distinct basement membranes, a parenchymal cell (astrocyte)-produced outer basement membrane and an endothelial cell- and pericyte-produced inner basement membrane, fuse to create a composite basement membrane in the mature BBB (Owens et al., 2008; Sixt et al., 2001). Basement membrane protein deficiency results in BBB disruption (Menezes et al., 2014). In addition, the basement membrane provides physical support for the cellular components of the BBB and anchors them in place (Cardoso et al., 2010).

The blood-CSF barrier, located at the choroid plexuses, separates the CSF from blood (Skipor and Thiery, 2008). The tight junctions form a barrier to prevent diffusion of circulating molecules through paracellular pathways in the blood-CSF barrier. However, they exist between choroidal epithelial cells, but not between endothelial cells.

Therefore, circulating molecules have to use specific transport systems to reach the brain parenchyma or CSF. Indeed, the endothelial cells of the BBB and choroidal epithelial cells of the blood-CSF barrier contain various transport systems that mediate the entry of hormones and nutrients (for reviews, see Banks, 2006; Daneman, 2012; Redzic, 2011; Skipor and Thiery, 2008). Transport through the choroid plexus permits circulating molecules to enter the CSF and to reach the Arc through the ventricular wall.

3. Vascular structure and cellular components of the Arc and ME

The Arc is morphologically located in close proximity to the ME, which lacks a typical BBB (Fig. 1), and accumulating evidence indicates that the Arc has access to circulating molecules through the ME (Cheunsuang et al., 2006; Faouzi et al., 2007). The ME contains fenestrated endothelial cells (Broadwell et al., 1983), which express a marker of endothelial fenestral diaphragms named plasmalemma vesicle protein-1 (Ciofi et al., 2009) but do not express tight junction proteins (Mullier et al., 2010; Norsted et al., 2008). These structural characteristics confer high permeability on this specific vasculature and make the ME a primary site of exchange between the systemic circulation and the brain. The high vascular permeability permits neuroendocrine neurons to secrete large amounts of hypothalamic releasing hormones into the bloodstream (Muller et al., 1999; Prevot et al., 2007). Another unique feature of the ME vasculature is the presence of a large perivascular space (Krisch and Leonhardt, 1978). In the ME, the basement membranes are separated into the outer and inner basement membranes, creating a large perivascular space outside the brain parenchyma.

Recently, we and Fredrich et al. have shown that continuous angiogenesis occurs in the ME (Fredrich et al., 2015; Morita et al., 2013). Developing brain vasculature undergoes angiogenesis and therefore possesses high vascular permeability, but angiogenesis is almost absent in the adult mammalian brain, except under pathological conditions such as injury or hypoxia (Hjelmeland et al., 2011). Brain endothelial cells are fenestrated during normal development. Fenestrations are reduced during late prenatal development and are almost entirely absent by birth (Hallmann et al., 1995). VEGF and its receptor VEGF receptor 2 (VEGFR2) are key regulators of angiogenesis. VEGF is highly expressed in somatodendrites, astrocytes, tanycytes and neuronal terminals, while VEGFR2 is expressed in the endothelial cells of the ME (Langlet et al., 2013; Morita et al., 2013). VEGF was originally found as a vascular permeability factor and is required for normal development of the embryonic vascular system (Greenberg and Jin, 2005). VEGF is also important for disrupting the BBB in autoimmune encephalomyelitis, a mouse model of multiple sclerosis (Argaw et al., 2009), and leukemia (Feng et al., 2011). VEGF down-regulates the tight junction proteins claudin-5 and occludin *in vitro*, and microinjection of VEGF disrupts claudin-5 and occludin expression and induces loss of barrier function (Argaw et al., 2009). VEGF induces tyrosine phosphorylation of endothelial adherens junction components (Esser et al., 1998) that may be involved in increased paracellular permeability. Brain-derived neurotrophic factor, which is also suggested to promote angiogenesis (Kermani and Hempstead, 2007), is expressed in astrocytes and tanycytes in the ME (Givalois et al., 2004).

Pericyte deficiency also increases the permeability of the BBB (Armulik et al., 2011; Daneman et al., 2010). NG2 chondroitin sulfate proteoglycan and platelet-derived growth factor receptor β (PDGFRβ) are prominent components of activated pericytes, and play important roles in pericyte recruitment and interaction with endothelial cells during BBB development (Fukushi et al., 2004; Ozerdem and Stallcup, 2004). While NG2 and PDGFRβ expression is down-regulated to some extent in pericytes associated with quiescent vessels, it becomes highly

up-regulated in pericytes in neovasculature associated with wound repair and tumors. NG2 and PDGFRβ are highly expressed in pericytes of the adult ME, indicating that the ME pericytes also possess immature or activated features (Morita et al., 2013). NG2 is additionally expressed by a class of glial cells called NG2-glia (Vigano and Dimou, 2016). NG2-glia may also be involved in angiogenesis in the ME, since they exist in the ME (Djogo et al., 2016) and are necessary for angiogenesis in the embryonic brain (Minocha et al., 2015). These data suggest that the ME vasculature is highly permeable because of its pro-angiogenic environment.

The ventromedial aspect of the Arc vasculature may be partially open to circulating molecules (Ciofi, 2011; Mullier et al., 2010; Norsted et al., 2008). Some vessels in the ventromedial Arc lack BBB markers such as endothelial-barrier antigen, transferrin receptor and glucose transporter GLUT-1, and express the endothelial fenestral diaphragm marker plasmalemmal vesicle-associated protein 1 (Ciofi et al., 2009; Norsted et al., 2008; Schaeffer et al., 2013). However, they also express the tight junction proteins claudin-5 and ZO-1, while the ME vasculature does not (Norsted et al., 2008), suggesting that the Arc vasculature is less permeable than the ME vasculature. Additionally, the ventromedial aspect of the Arc vasculature is connected with the ME vasculature (Ambach et al., 1976).

4. Access of circulating molecules from the ME to the Arc

Vascular permeability of the Arc and ME has been analyzed using tracer molecules of various sizes. When radioiodinated α-aminoisobutyric acid (^{14}C-α-aminoisobutyric acid; molecular weight (MW) = 103) was injected into the tail vein of living rats, tracer became distributed in the Arc and ME (Shaver et al., 1992). Other circulating tracers such as fluorescein (MW = 332) (Natah et al., 2005), fluorescein isothiocyanate (FITC; MW = 389) (Morita and Miyata, 2012, 2013), Evans blue (MW = 961) (Morita and Miyata, 2012, 2013), and dextran 3000 (MW = 3000) (Morita and Miyata, 2013) also became distributed in the Arc and ME.

To investigate whether these tracers spread by passive diffusion or active transport, we administered Evans blue and FITC tracers to mice fixed with paraformaldehyde. Their fluorescence generated an intensity gradient emerging from the fenestrated capillary existing in the external zone of the ME (Fig. 2a) (Morita and Miyata, 2013), indicating that these tracers have access to the external zone of the ME by passive diffusion. In contrast, we did not detect fluorescence of tracers in the Arc or in the ependymal and internal zones of the ME (Fig. 2a) (Morita and Miyata, 2013).

Some alternative diffusion barriers are proposed in lieu of the BBB. Tanycytes are glia-like polarized cells located around the base of the third ventricle in the hypothalamus (Fig. 3) (Rodriguez et al., 2005). One subset of tanycytes, β1-tanycytes, probably prevent parenchymal passive diffusion of molecules from the ME to the Arc. They line the border between ME and Arc and prevent parenchymal diffusion of tracers (Campbell et al., 2017; Krisch and Leonhardt, 1978; Rethelyi, 1984; Rodriguez et al., 1979, 2005), although tight junction proteins are not observed in their processes (Mullier et al., 2010; Petrov et al., 1994; Smith and Shine, 1992). β2-Tanycytes, on the other hand, express tight junction proteins to form a ME-CSF barrier (Mullier et al., 2010). When Evans blue was injected into the lateral ventricle, it did not spread into the ME (Mullier et al., 2010). Astrocytes are mainly distributed in the internal zone of the ME just below the ependymal layer, and they have perivascular processes in the ME (Tamada et al., 1997). Astrocytes form a barrier in other circumventricular organs including the organum vasculosum of the lamina terminalis, subfornical organ, and area postrema (Morita et al., 2016). Moreover, some extracellular matrix components, such as brevican and neurocan, decorate the internal zone of the ME (Pocsai and Kalman, 2014). The extracellular matrix probably represents a passive diffusion barrier for the node of Ranvier and AMPA receptors on dendrites (Bekku et al., 2010; Frischknecht et al., 2009). These tanycytes and astrocytes, and along with the extracellular matrix, may act as a barrier separating the ME and Arc.

Larger blood-derived tracers such as dextran 10,000 (MW = 10,000) accumulated within the perivascular space in the external zone of the ME (Fig. 2b and c) (Morita and Miyata, 2013). The permeability of tracers gradually decreases as their MW increases, and little or no tracer diffuses into the parenchyma when the MW exceeds 40,000 (Schaeffer et al., 2013). The molecular weights of hypothalamic releasing hormones that are released from the ME to the blood range from 362 to 5040 (thyrotropin-releasing hormone: MW = 362; somatostatin: MW = 1638; corticotropin-releasing hormone: MW = 4758; gonadotropin-releasing hormone: MW = 1182; growth hormone-releasing hormone: MW = 5040). Considering the molecular weights of these hormones, the ME vasculature does not need to be penetrable by large molecules.

When horseradish peroxidase (MW = 44,000) and dextran 70,000 (MW = 70,000) were injected into the tail vein of living mice, they were distributed in the Arc (Broadwell et al., 1983; Herde et al., 2011; Morita and Miyata, 2012). Endogenous IgG (MW = 150,000) was also found in the Arc (Natah et al., 2005). FITC-BSA (MW = ~66,000–70,000) reached the Arc 120 min after tracer injection, whereas it was faint 10 min after the injection (Fig. 2h and i) (Morita and Miyata, 2012). Moreover, when we injected Evans blue and FITC tracers into living mice, noticeable fluorescence was found in nestin-positive tanycytes and microtubule associated protein 2-positive neuronal somata in the ependymal/internal zone of the ME and Arc (Fig. 2d–g) (Morita and Miyata, 2013). The intracerebroventricular administration of a lectin, wheat germ agglutinin (MW = 38,000), results in the accumulation of the tracer molecule in the cell body of tanycytes and the proximal segment of their basal processes at the ependymal zone in the ME and Arc (Peruzzo et al., 2004; Rodríguez et al., 2010). Wheat germ agglutinin was taken up by tanycytes in the Arc via cellular internalization (Peruzzo et al., 2004).

In the ME, β2-tanycytes bridge between the third ventricle and the vasculature, directly contacting the CSF at their apical surface and with their end-feet adjacent to the perivascular space, to provide an interconnection between the CSF and circulation (Peruzzo et al., 2004; Rodriguez et al., 2005; Wagner and Pilgrim, 1974). Accumulating evidence suggests that β2-tanycytes actively transport circulating molecules from the ME vasculature to the CSF, via transcellular vesicle-mediated transport, in which molecules are taken up on one side by endocytosis and moved to the opposite side where they are released by exocytosis (Balland et al., 2014; Fernandez-Galaz et al., 1996; Peruzzo et al., 2004). Tanycytes possess microvilli, which are needed for transcytosis, and proteins involved in endocytosis and transcytosis such as clathrin, caveolin-1, Rab4, and ARF6 (Peruzzo et al., 2004). Once they reach the CSF, circulating molecules may be transported by α2-tanycytes located at the level of the Arc or diffuse into the Arc parenchyma because α2-tanycytes form a permeable layer without an intact tight junction component (Mullier et al., 2010). These results indicate that the ability of circulating molecules to access the ependymal/internal zone of the ME and Arc depends on active transport by tanycytes rather than diffusion from a fenestrated capillary.

Some Arc neurons project their axons and terminate in the ME. If such axon terminals have receptors for hormones and nutrients and retrogradely transport them, the Arc neurons might directly access these molecules. Distribution of the ghrelin receptor has been investigated only at the mRNA level, so whether the receptor itself is present in the neuronal terminals is unclear (Bennett et al., 1997). Peripherally injected radioiodinated insulin (^{125}I-insulin) binds terminals of Arc neurons in the ME, indicating that there are insulin-receptive nerve terminals there (van Houten et al., 1980, 1983). Although the cellular localization remains unclear, insulin receptor was also found in the ME (Unger et al., 1989). In addition, a recent study demonstrated that there are leptin receptor-positive dendrites of Arc

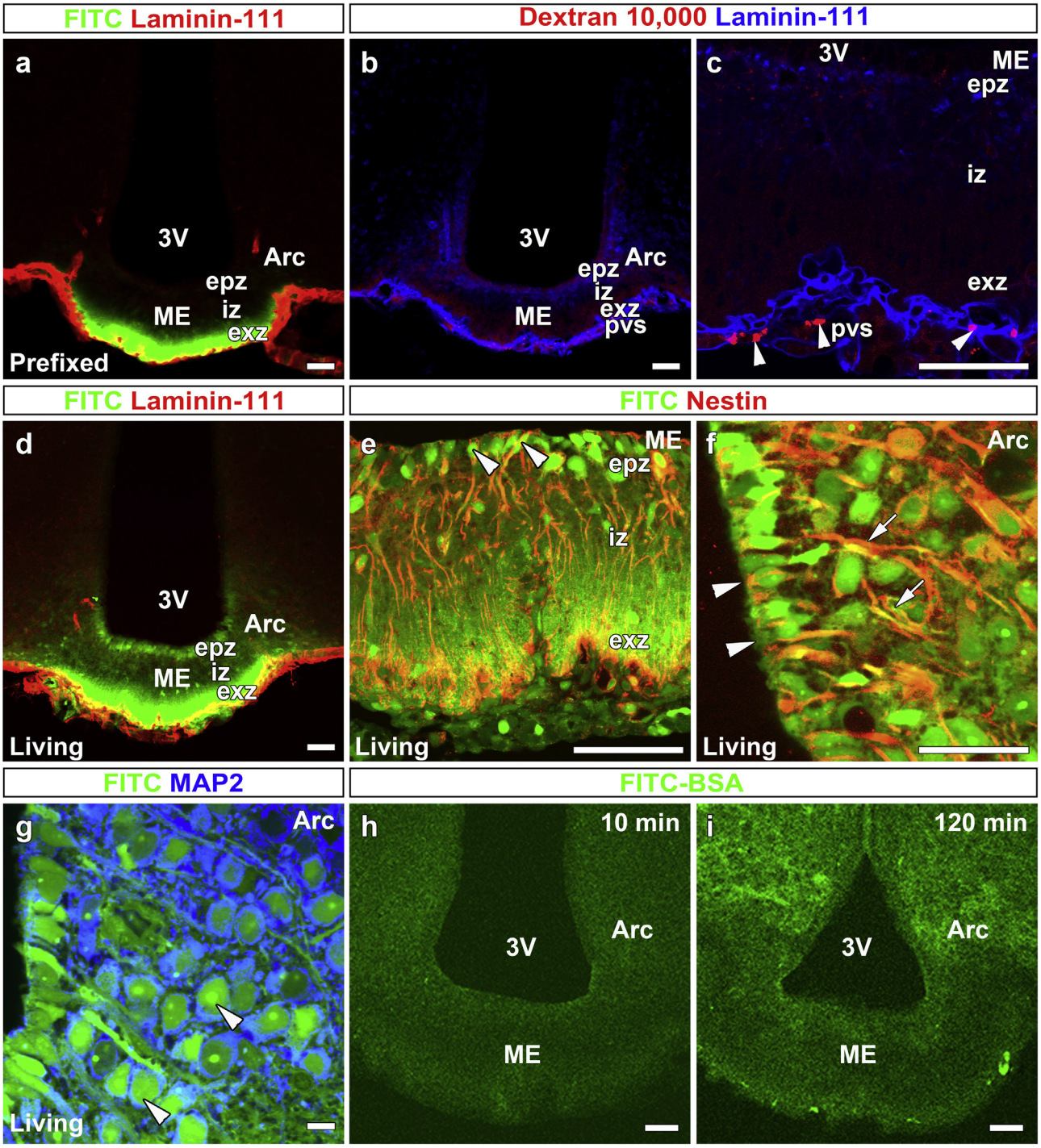

Fig. 2. Access of circulating molecules to the ME and Arc. Cryosections were immunostained with antibodies against the outer basement membrane marker laminin-111, the tanycyte marker nestin, or the neuron marker microtubule associated protein 2 (MAP2). (a) Distribution of transcardially perfused FITC. Mice were fixed before perfusion. (b,c) Distribution of intravenously injected dextran 10,000 (arrowheads). Mice were fixed 30 min after injection. (d–g) Distribution of transcardially perfused FITC in cell bodies (arrowheads) and cellular processes (arrows) of tanycytes (e,f) and neurons (g). Mice were fixed just after perfusion. (h,i) Distribution of intravenously injected FITC-BSA. Mice were fixed 10 or 120 min after injection. 3 V: third ventricle; epz: ependymal zone; exz: external zone; iz: internal zone; pvs: perivascular space. Scale bars = 50 μm. This figure is adapted from Morita and Miyata (2013) (reprinted with permission of John Wiley and Sons) and Morita and Miyata (2012) (reprinted with permission of Springer Nature).

neurons in the ME, and that their degeneration results in hyporesponsiveness to circulating leptin, indicating that these Arc neurons directly sense leptin in the ME (Djogo et al., 2016). We summarize the access of circulating molecules from the ME to the Arc in Fig. 3.

5. Hormones and nutrients that reach the Arc

Circulating metabolic hormones and nutrients are known to act on the Arc neurons, but how they reach these neurons is still being debated. Elucidation of the mechanisms underlying these molecules' entry into the Arc is important for understanding not only normal

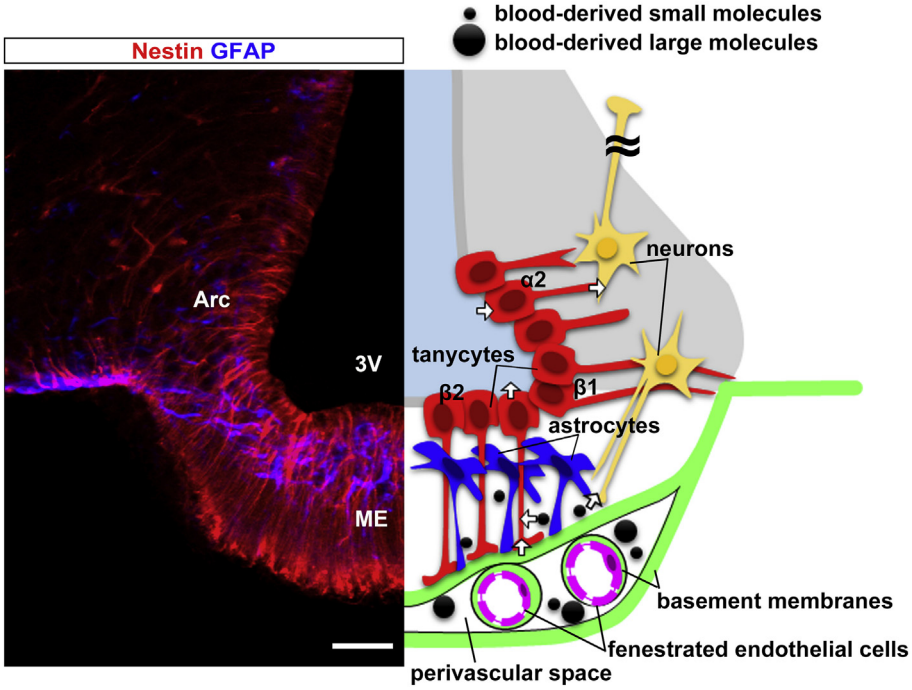

Fig. 3. Schematic representation of the access of circulating molecules from the ME to the Arc. The cryosection on the left was immunostained with antibodies against the tanycyte marker nestin and the tanycyte/astrocyte marker GFAP. α-Tanycytes and β-tanycytes are nestin-positive, whereas dorsal α-tanycytes and astrocytes, but not ventral α- and β-tanycytes, are GFAP-positive. Arrows on the right indicate the direction of circulating molecules. 3 V: third ventricle. Scale bar = 50 μm.

maintenance of homeostasis but also how this is impaired in pathologies such as obesity and diabetes. We cannot explain the entry of each hormone and nutrient solely in terms of their molecular weight, but vascular permeability analysis using tracers indicates size-dependent passive diffusion from the ME vasculature and active transport by tanycytes from the ME to the Arc. In this section, we will summarize the access of circulating metabolic hormones and nutrients to the Arc.

5.1. Leptin

Leptin (MW = 16,000), a satiety hormone, is mainly produced by adipocytes and secreted from adipose tissue into the circulation (for a review, see Hwang et al., 1997). Circulating leptin accesses some neurons in the Arc to induce satiety and control food intake (Faouzi et al., 2007). The ME and choroid plexus was labeled with intravenously injected radioiodinated leptin (^{125}I-leptin) and intraperitoneally injected fluorescently labeled leptin, indicating that leptin reaches cells in the Arc via the ME and choroid plexus (Banks et al., 1996; Harrison et al., 2018; Karonen et al., 1998). The ME tanycytes can actively transport leptin into the Arc (Balland et al., 2014). Peripherally injected leptin binds tanycytic processes and activates its receptor in the tanycytes to be transported to the CSF. In view of its molecular weight, passive diffusion of leptin from the ME vasculature is likely to be impeded to some extent. Indeed, leptin signaling pathways in the hypothalamus were not maximally activated until 1 h after an intraperitoneal leptin injection (Faouzi et al., 2007). This time-lag disappears when leptin is intracerebroventricularly administered (Faouzi et al., 2007), suggesting that leptin is actively transported to the Arc. Another study showed that Arc neurons projecting into the ME express the leptin receptor, and their degeneration results in hyporesponsiveness to circulating leptin (Djogo et al., 2016). Leptin is also transported across the BBB and blood-CSF barrier to control food reward (Burguera et al., 2000; Di Spiezio et al., 2018; Skipor and Thiery, 2008; Zlokovic et al., 2000).

5.2. Ghrelin

The orexigenic hormone ghrelin (MW = 3371) induces food intake by directly acting on NPY/AgRP neurons in the Arc (Kohno et al., 2003; Nakazato et al., 2001). The mechanism underlying the access of ghrelin, which is produced and secreted in the stomach (Kojima et al., 1999), to the Arc is not yet fully understood, but recent work indicates that ghrelin is also internalized by tanycytes (Collden et al., 2015). In another study using a real-time *in vivo* imaging method, peripherally injected ghrelin diffused through the fenestrated vasculature of the ME (Schaeffer et al., 2013). As ghrelin is small enough to passively diffuse from the ME vasculature, it would reach the ME parenchyma, be transported by β2-tanycytes into the CSF, and then reach the Arc. Because neurons were rapidly labeled with circulating ghrelin in the ventromedial Arc, ghrelin is also presumed to diffuse from the ventromedial Arc vasculature (Schaeffer et al., 2013).

5.3. Insulin

Circulating insulin (MW = 5807) binds to receptors located in the Arc (Marks et al., 1990; Unger et al., 1989; van Houten et al., 1980; Werther et al., 1987). Insulin is a pancreatic hormone that is directly involved in glucose metabolism and homeostasis. Within the brain, it acts to increase energy expenditure and reduce food intake and energy storage (for a review, see Porte et al., 2005). The mechanism by which circulating insulin reaches the Arc neurons is also unclear, but, given its molecular weight, insulin probably passively diffuses from the ME vasculature. Peripherally injected radioiodinated insulin (^{125}I-insulin) binds to nerve terminals in the ME, indicating that there are insulin-receptive nerve terminals in the ME (van Houten et al., 1980). The ME tanycytes may transport insulin to the CSF in the same way as ghrelin. Insulin is also transported across the BBB in an insulin receptor-independent manner (Rhea et al., 2018).

5.4. Others

Although it is unclear how these circulating molecules enter the Arc, some other hormones including peptide YY (MW = 4,050, Batterham et al., 2002), glucagon-like peptide-1 (GLP-1) (MW = 3,298, Secher et al., 2014), glucocorticoids (cortisol MW = 363, Savontaus et al., 2002), estrogens (estradiol MW = 272, Gao et al., 2007) and prolactin (MW = 23,000, Brown et al., 2010) also act on Arc neurons. Peptide YY is released by gut cells and acts on these neurons, presumably by

inhibition of NPY/AgRP neurons, to reduce food intake (Acuna-Goycolea and van den Pol, 2005). GLP-1 is produced by L-cells of the ileum and is secreted during meal ingestion. A GLP-1 receptor analog is internalized into Arc POMC/CART neurons *in vivo*, and GLP-1 directly stimulates POMC/CART neurons and indirectly inhibits neurotransmission in NPY/AgRP neurons *in vitro* (Secher et al., 2014). Its receptors, which are highly expressed in the POMC/CART neurons of the Arc, are involved in the maintenance of glucose homeostasis (Sandoval et al., 2008). Estrogen receptor is also expressed in Arc POMC/CART neurons (de Souza et al., 2011), and the expression of leptin receptor is regulated during the estrous cycle in the Arc (Bennett et al., 1999). Local administration of a synthetic glucocorticoid dexamethasone in the Arc induces severe hepatic insulin resistance, suggesting that glucocorticoid signaling in the Arc modulates hepatic insulin sensitivity (Yi et al., 2012a). Prolactin receptor is expressed in dopamine neurons of the Arc, and binding of prolactin to the neurons suppresses the production of prolactin (Kokay and Grattan, 2005).

While nutrients such as glucose and fatty acids enter the brain by crossing the BBB through their specific transporters to provide cellular energy (for reviews, see Mitchell and Hatch, 2011; Patching, 2017), accumulating evidence indicates that the hypothalamus, including the Arc, senses glucose and fatty acids to maintain energy balance. Glucose-sensing neurons exist in the Arc (Fioramonti et al., 2007). As the MW of glucose is 180, it may diffuse to the external zone of the ME and then be transported by tanycytes. Indeed, the glucose transporters GLUT1 and GLUT2 are expressed in α- and β-tanycytes, and are functional in cultured tanycytes (for a review, see Bolborea and Dale, 2013; Garcia et al., 2003). NPY mRNA expression decreased after intracerebroventricular administration of long-chain fatty acids (oleic acid), and oleic acid excites POMC neurons (Jo et al., 2009), indicating that fatty acids act on the Arc neurons (Obici et al., 2002). Moreover, tanycytes show higher levels of lipid droplet content in diet-induced obese mice than in normal diet-fed mice, indicating that tanycytes contribute to lipid sensing in the Arc (Hofmann et al., 2017).

6. Structural remodeling of the Arc and ME in response to fluctuating energy balance

Accessibility of the Arc to circulating molecules is not constant. Although diffusion of circulating molecules from the vasculature in the Arc is prevented in fed mice, it does occur in fasted mice via VEGF-dependent increases in vascular fenestration and permeability of the Arc and ME (Langlet et al., 2013). This may be why the ME not only lacks a typical BBB, but also displays angiogenic behavior to modify its vascular structure and permeability in response to fluctuating energy balance. Angiogenesis and hypervascularization also occur in the Arc of mice fed with a high calorie diet, and increased numbers of pre-capillary arterioles were found in the infundibular nuclei, equivalent to the Arc of rodents, of patients with type 2 diabetes mellitus (Yi et al., 2012b). Ghrelin transport, presumably by tanycytes, is down-regulated when neonatal mice are overfed (Collden et al., 2015). Moreover, diet-induced obesity in adult mice attenuates the ability of the ME tanycytes to transport leptin (Balland et al., 2014). It is conceivable that if this blood-to-Arc communication through the ME is impaired in pathological conditions such as obesity and diabetes, circulating metabolic hormones and nutrients become unable to reach the Arc neurons appropriately.

Not only angiogenesis, but also neurogenesis and gliogenesis (NG2-glia, astrocytes, microglia, and oligodendrocytes) occur in the ME of adult rodents (Bennett et al., 2009; Fredrich et al., 2015; Virard et al., 2008), and continuous neurogenesis was also detected in the ME and infundibular nucleus (Arc) of adult human brains (Batailler et al., 2014; Sanin et al., 2013). When ciliary neurotrophic factor was injected into the lateral ventricle, neurogenesis was enhanced in the hypothalamus including the Arc, and body weight decreased in adult mice, while the mitotic inhibitor cytosine-b-D-arabinofuranoside eliminated the neurogenesis and abolished the long-term effects of ciliary neurotrophic factor on body weight (Kokoeva et al., 2005). The inhibition of neurogenesis from β2-tanycytes attenuates weight gain in high-fat diet-fed mice (Lee et al., 2012). Neurogenesis was attenuated in the Arc of diet-induced obese mice or leptin-deficient mice (McNay et al., 2012). α2-Tanycytes are able to self-renew or give rise to β2-tanycytes and parenchyma cells *in vivo* and exhibit stem-like neurospherogenic activity *in vitro* (Robins et al., 2013). These findings support the idea that the Arc and ME adapt their structure including vasculature, neurons and glia in response to fluctuating energy balance.

7. Concluding remarks

The ME vasculature not only lacks a typical BBB, but also displays angiogenic behavior and size-dependent permeability. Neuronal cell bodies in the Arc are protected from diffusion of circulating molecules from the ME vasculature. Active transport by tanycytes through the CSF enables Arc neurons to sense circulating molecules. Structural plasticity of the Arc and ME contributes to maintaining energy homeostasis. Thus, the ME is not just an area that allows passive diffusion of circulating molecules via the leaky fenestrated vasculature, but should also be considered a dynamic gateway to the Arc for circulating molecules. Future studies are warranted to fully delineate the relative contributions of the ME, Arc, BBB and blood-CSF barrier in transport of circulating metabolic hormones and nutrients to the Arc neurons.

Conflicts of interest

The authors declare no conflict of interest.

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