DHA (omega-3 fatty acid) and estradiol: key roles in regional cerebral glucose uptake

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Abstract – Neurons have a high energy need, requiring a continuous supply of glucose from the blood. Tight regulation of glucose metabolism in response to stimuli is essential for brain physiology. Glucose metabolism and cerebral blood flow are closely coordinated during neuronal activity to maintain proper brain function. Glucose uptake across the blood-brain barrier is facilitated by a carrier protein: the GLUT-1 transporter. The first way the body meets urgent demand for glucose is to increase the blood flow through vasodilatory responses generated by nitric oxide. If that is insufficient, the second way is to increase the density of GLUT-1 through the translocation of this transporter from intracellular stores. The third pathway is to increase GLUT-1 synthesis by stimulating SLC2A1 (GLUT-1 gene) transcription. A tandem of two key molecules, free estradiol and DHA, is involved in this critical regulation. Their relationship is synergistic and reciprocal: free estradiol with genomic and non-genomic actions via ERα, and DHA via the PPARα-RXRα and PPARγ-RXRα heterodimers. We highlight several original mechanisms linking two main principles (neuronal stimulation and brain energy metabolism) with the fundamental roles played by DHA and free estradiol. In particular, it has been shown that from a certain level of chronic DHA deficiency, a permanent imbalance sets in with disturbances in glucose intake and brain metabolism. This DHA deficiency is an aggravating factor in some neuropathologies.

Keywords: Brain / glucose / DHA / estradiol / GLUT-1

Résumé – DHA (acide gras oméga-3) : un rôle clé dans l’absorption cérébrale du glucose. Dans le cerveau, les neurones présentent la plus forte demande énergétique et nécessitent un apport continu de glucose par le sang. Une régulation étroite du métabolisme du glucose, en réponse à des stimuli, est essentielle. Au cours de l’activité neuronale, le métabolisme du glucose et le flux sanguin cérébral sont étroitement coordonnés pour maintenir une fonction cérébrale appropriée. L’absorption du glucose à travers la barrière hémato-encéphalique est facilitée par le transporteur GLUT-1. La première façon de répondre aux demandes urgentes de glucose est d’augmenter le flux sanguin par le biais d’actions vasodilatatrices générées par le monoxyde d’azote. La deuxième consiste à augmenter la densité de GLUT-1 par translocation de ce transporteur à partir des réserves intracellulaires. La troisième consiste à augmenter la synthèse du GLUT-1 en stimulant la transcription du SLC2A1 (GLUT-1 gène). Un tandem de deux molécules clés, l’estradiol libre et le DHA, est impliqué dans cette régulation. Leur interrelation est synergique et réciproque : l’estradiol libre avec des actions génomiques et non génomiques via ERα, et le DHA via les hétérodimères PPARα-RXRα et PPARγ-RXRα. Avec cet article, nous mettons en évidence plusieurs mécanismes originaux reliant deux grands principes : la stimulation neuronale et le métabolisme énergétique cérébral, avec les rôles fondamentaux joués par le DHA et l’estradiol libre. En particulier, il montre qu’à partir d’un certain niveau de carence chronique en DHA, un déséquilibre permanent s’installe avec une perturbation de l’apport en glucose et du métabolisme cérébral. Cette carence en DHA est un facteur aggravant de certaines neuropathologies.

Mots clés : Cerveau / glucose / DHA / estradiol / GLUT-1

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1 Introduction

The mammalian brain depends on glucose as its primary source of energy. Neurons have the highest energy requirements of all of the human brain’s components (Howarth et al., 2012), requiring a continuous supply of glucose from the blood. Glucose metabolism provides energy for physiological brain function in the form of ATP, the basis for neuronal and non-neuronal cell maintenance, as well as through the production of neurotransmitters. Therefore, tight regulation of glucose metabolism in response to stimuli is essential for brain physiology. Disruption of this metabolism in the brain is the cause of several diseases affecting both the brain itself and the entire organism (Mergenthaler et al., 2013).

Glucose metabolism and cerebral blood flow are closely coordinated during neuronal activity, to maintain proper brain function. Glucose transport across the blood-brain barrier (BBB) is facilitated by a single carrier protein, the GLUT-1 (glucose transporter). The delivery of this energy source is modulated by cerebral blood flow, which is one of the important factors in the regulation of brain function. The critical importance of controlling regional cerebral blood flow was reported as early as 1890 in a landmark publication (Roy et al., 1890). Both regional cerebral blood flow and regional cerebral glucose utilization are reliable indices of synaptic function (Jueptner et al., 1995). Astrocytes play a key role in the regulation of regional cerebral blood flow and glucose uptake (Paulson et al., 2010). An increase in synaptic activity leads to a dilation of local parenchymal arterioles that corresponds to the increase in metabolic demand. Cerebral blood flow and vascular smooth muscle tone are regulated by nitric oxide (NO) (Moncada et al., 1991). The nitric oxide pathway is the first pathway for meeting urgent demand for glucose, as well as other nutrients, such as other nutrients, DHA (docosahexaenoic acid), ALA (linolenic acid), EPA (eicosapentaenoic acid), testosterone, estradiol, IGF-1, oxygen, etc. The second pathway for increasing glucose intake, as well as DHAA (dehydroascorbic acid), is to increase the density of GLUT-1 through the translocation of this transporter from intracellular stores. The third pathway is to increase GLUT-1 synthesis by stimulating SLC2A1 (GLUT-1 gene) transcription (Majou, 2015). All steps in the process are complementary and highly regulated.

For glutamatergic neurons, studies have provided insight into the functional implications of the fundamental role of glucose metabolism in physiological and pathological brain function. In this opinion article, we highlight several original concepts linking two main principles: neuronal stimulation and brain energy metabolism, with the fundamental and synergic roles played by the DHA-estradiol tandem.

2 Neuronal metabolism: sources of energy

2.1 Energy in the form of ATP

ATP provides energy for the chemical reactions involved in metabolism through hydrolysis. ATP is a coenzyme for the transfer of phosphate groups associated with the kinases non-covalently. It cannot be stored in a raw state and is produced continuously. The body’s ATP stocks do not exceed more than a few seconds of consumption. Important pathways by which eukaryotes generate ATP are the glycolysis (Embden-Meyerhof-Parnas pathway), the citric acid cycle (or the Kreb’s cycle), and the electron transport chain (or the oxidative phosphorylation pathway). Together, these three stages are referred to as cellular respiration. Glycolysis is the metabolic pathway that converts glucose via a series of reactions to pyruvate. Pyruvate undergoes oxidative decarboxylation to produce acetyl-CoA. The acetyl-CoA is used as a substrate for the Kreb’s cycle, which occurs in the mitochondria (Fig. 1).

2.2 The astrocyte-neuron lactate shuttle

Lactate is a ubiquitous metabolite that originates from glycolysis. Its role as an energy substrate in the brain, alongside glucose, has also been highlighted. Over the last 25 yr or so, the astrocyte-neuron lactate shuttle (ANLS) concept (Magistretti, 1997; Pellerin et al., 2003) has become widely interpreted as meaning that the neuron’s principal source of energy seems to be lactate from glucose. This process proposes that astrocytes play the key role in the coupling of neuronal activity and cerebral glucose utilization. According to this concept, astrocytes are the primary consumer of bloodborne glucose in the brain. Within astrocytes, glucose is metabolized glycolytically to lactate, which is then exported as the neuron’s primary source of energy; this process is stimulated by glutamate. Astrocytes have a key role in maintaining the functionality of synapses and neuronal circuits, and store glucose in the form of glycogen (Rodriguez et al., 2009). Astrocyte-to-neuron signaling in intact tissue contributes to synaptic plasticity (Fiacco et al., 2004).

The two pyruvate molecules produced from one glucose molecule have two uses in astrocytes: (i) the reduction in lactate catalyzed by lactate dehydrogenase (LDH) (O’Brien et al., 2007), (ii) their use as a substrate of the Kreb’s cycle to enable the continued production of ATP molecules through oxidative phosphorylation (Pellerin et al., 2003; Fig. 1). Astrocytes and neurons have different LDH isoforms: LDH5 is the main isoform expressed in astrocytes, and it converts pyruvate to lactate whereas LDH1 is the major isoform in neurons to convert lactate to pyruvate. The rate of pyruvate production exceeds the catalytic activity of pyruvate dehydrogenase, and lactate production occurs. The enzymatic activity of LDH is much higher than that of the pyruvate dehydrogenase.

Astrocytic lactate is an intermediate metabolite of energy storage in neurons. It is an element of the elasticity of energy metabolism. It compensates for the difference in speed between the pathways of glycolysis and aerobic production of ATP. At rest, neurons spend most of their energy production in aerobic glycolysis (>90%) for the maintenance of transmembrane ion concentration gradients through Na⁺/K⁺-ATPase. Aerobic glycolysis saturates quickly during prolonged stimulation (Fox et al., 1988). A second energy pathway must quickly replace the first one to secure neuronal and synaptic function upon stimulation. This alternative route involves two main intermediaries: astrocytes and lactate. The transient formation of lactate during stimulation has been demonstrated in several studies (Prichard et al., 1991; Sappey-Marinier et al., 1992; Fellows et al., 1993).
3 Sustaining ATP demand caused by glutamatergic stimulation

At the astrocyte, the flow of glucose, its concentration and the kinetics of ATP production are dependent on the stimulation mediated by glutamate for glutamatergic neurons with a pre- and postsynaptic synchronization (Magistretti, 2009). Glutamate is released into the synaptic cleft and activates ionotropic glutamatergic receptors, producing a postsynaptic depolarization. Astrocytic excitatory amino acid transporters (EAATs) are responsible for the uptake of a large fraction of glutamate at the synapse and they control glutamate homeostasis. EAAT2, which is concentrated in perisynaptic astrocytes, performs 90% of glutamate uptake. Glutamate is converted into glutamine by glutamine synthetase and shuttled back to neurons for glutamate synthesis (Allaman et al., 2011). The glutamate-glutamine shuttle consumes two ATP molecules: one molecule of ATP for astrocytes to capture glutamate through the action of the Na⁺/K⁺-ATPase (Magistretti et al., 1997; Schurr et al., 1998), and one molecule of ATP to convert the glutamate to glutamine by glutamine synthase (Smith et al., 1991). This energy consumption activates astrocytic glycolysis, its enzymatic chain and phosphorylation of glucose to glucose-6-phosphate by hexokinase to find equilibrium a balance between osmotic input and consumption.

In order to meet this need for glucose, different processes are mobilized according to different spatiotemporal modalities. The first way the body meets urgent demand for glucose is to increase the blood flow through vasodilatory responses generated by nitric oxide. If that is insufficient, the second way is to increase the density of GLUT-1 through the translocation of this transporter from intracellular stores. The third pathway is to increase GLUT-1 synthesis by stimulating SLC2A1 (GLUT-1 gene) transcription.

4 Vasodilatation and glucose uptake

Nitric oxide (NO), produced by many cells in the body, relaxes vascular smooth muscle. It is produced by a group of enzymes called nitric oxide synthases (NOS). Three NOS isoforms have been identified: neuronal NOS (nNOS or NOS 1), endothelial NOS (eNOS or NOS 3), and an inducible NOS (iNOS or NOS 2). The expression of the iNOS is one of the direct consequences of an inflammatory process. iNOS is induced in astrocytes and microglia under pathological conditions; NOS are localized in synaptic spines, astrocytes, and the loose connective tissue surrounding blood vessels in the brain; eNOS are present in cerebral vascular endothelial cells, motor neurons, dendritic spines (Caviedes et al., 2017) and astrocytes (Wienczen et al., 1999). During physiological processes, NO produced by both eNOS and
nNOS controls blood flow activation through vasodilatory responses \cite{Reis2017}. eNOS become more prominent at lower levels of neuronal activity and nNOS dominate at higher neuronal activation levels \cite{deLabra2009}. As the intensity of stimulation increases (glutamate, Ca$^{2+}$), there is a parallel increment of NO production. Thus, eNOS is active at normal levels of stimulation; when levels of activity increase, there is a switch from eNOS to nNOS.

These enzymes, activated by phosphorylation, convert arginine into citrulline, producing NO in the process. Oxygen and NADPH are necessary co-factors. The phosphorylation of eNOS resembles that of AS160 \cite{Schneider2003} via the activation/phosphorylation of AMP-activated protein kinase (AMPK) or via the PI3K (phosphoinositide-3-kinase)/Akt signaling pathway (Fig. 2). The first one, via AMPK, is induced most commonly upon activation of the NMDA receptor (N-methyl-D-aspartate) subtype of the glutamate receptor, which results in calcium influx \cite{Zonta2003,Stobart2013}. Ca$^{2+}$ binds to calmodulin-independent protein kinase II (CaMKII), which phosphorylates and activates eNOS \cite{Schneider2003}. In nNOS and eNOS, calmodulin binding is brought about by an increase in intracellular Ca$^{2+}$. When calmodulin affinity to NO increases, it facilitates the flow of electrons from NADPH in the reductase domain to the haem in the oxygenase domain.

Activation of eNOS and nNOS is thought to depend on the phosphorylation of serine 847 and serine 1412. A low glutamate concentration (30 μM glutamate) induces rapid and transient NMDA receptor-dependent phosphorylation of S1412 by Akt, followed by sustained phosphorylation of S847 by CaMKII of nNOS \cite{Rameau2007}. But the phosphorylation of nNOS at S847 by CaMKII attenuates the catalytic activity of the enzyme \cite{Komeima2000}. Moreover, an excitotoxic stimulus (150 μM glutamate) induced S1412, but not S847 phosphorylation of nNOS \cite{Rameau2007}. In concert with CaMKII signaling in the post-translational activation of eNOS, the LKB1/AMPK/eNOS pathway is also activated by the depletion of ATP \cite{Vázquez-Chantada2009;Fig. 2}. In addition, MAPK pathway (mitogen-activated protein kinase) works in synergy with and activates the PI3K/Akt signaling pathway (see mechanism below).

NO diffuses freely across membranes (it is a transient paracrine and autocrine signaling molecule) into smooth muscle cells surrounding arterioles. There it activates soluble guanylyl cyclase (sGC) (a heterodimer with a heme moiety as a prosthetic group) yielding cyclic GMP (cGMP) from guanosine triphosphate (GTP). The binding of NO to the ferrous (Fe$^{2+}$) center of the heme moiety induces a conformational change and leads to a several hundred-fold increase in cGMP.
5.1 Glucose and the fundamental role of GLUT-1

Glucose is transported across the BBB from the blood into the brain intercellular space. Intercellular glucose is then transported into neurons and astrocytes via a process of facilitated diffusion. Once the substrate is transported into the brain intercellular space, intercellular glucose is then transported into neurons and astrocytes (Ulatowski et al., 2022), inhibiting lipid peroxidation, thus preventing membrane damage and the modification of low-density lipoproteins. Moreover, vitamin E and vitamin C work together to protect vulnerable polyunsaturated fatty acids such as omega-3s. And, as we shall see later, an omega-3 fatty acid, docosahexaenoic acid (DHA), is a key molecule in GLUT-1 transporter function. In fact, brain tissue is especially sensitive to oxidative injury because of its higher metabolic rate driven by glucose and oxygen (reactive oxygen species are generated continuously during oxidative metabolism), lower concentrations of protective antioxidants, and higher levels of polyunsaturated fatty acids that are susceptible to lipid peroxidation (Markesbery, 1999). The most reactive forms, such as the hydroxyl radical, are also capable of oxidizing proteins and nucleic acids.

Whereas most mammals synthesize L-ascorbic acid de novo in their liver, anthropoid primates, including humans, certain bats and guinea pigs, are incapable of doing so. This is due to a mutation in the GULO gene (GULO gene), thought to have occurred during the late Eocene (Majou, 2018). These animals have an inactive form of the altered GULO gene (GULO pseudogene), which does not allow the enzyme to be synthesized (Ohta et al., 1999). They must therefore regularly obtain it from dietary sources in oxidized form (DHA). L-ascorbic acid is not transported across the capillary endothelial cells in the blood-brain barrier. DHA is transported through the blood-brain barrier by GLUT-1 transporters (Rumsey et al., 1997) and then immediately converted into L-ascorbic acid by enzymes, namely NADPH-dependent thioredoxin reductase, glutathione-dependent protein disulfide isomerase, and DHAA reductase (Agus et al., 1997), particularly in astrocytes.

5.2 GLUT-1, transporter of dehydroascorbic acid (DHAA), and antioxidant defenses

Nerve endings in the brain contain the highest concentrations of L-ascorbic acid in the human body after the suprarenal and pituitary glands (Bourre, 2006). This concentration in the brain exceeds blood concentrations by at least tenfold. As an electron donor, L-ascorbic acid (vitamin C) has an antioxidant function in the brain. During reduction of free radicals, L-ascorbic acid is oxidized to dehydroascorbic acid (DHAA) by giving two electrons. DHAA, is reduced by glutathione, then the oxidized glutathione is reduced by the action of glutathione reductase in a reaction coupled with NADPH. The glutathione/oxidized glutathione redox state is coupled with the L-ascorbic acid/DHAA redox state by both enzymatic and non-enzymatic processes (Harrison et al., 2009).

Working in synergy with the activity of the main free radical scavenging enzymes, copper/zinc superoxide dismutase, manganese superoxide dismutase, catalase, and glutathione peroxidase, L-ascorbic acid protects cellular components from free radical damage. It scavenges free radicals directly in the aqueous phases of cells and the circulatory system. L-ascorbic acid also protects membrane and other hydrophobic compartments from such damage by regenerating the antioxidant form of dietary vitamin E (Beyer, 1994). Vitamin E, localizes to lysosomes and mitochondria in primary neurons and astrocytes (Ulatowski et al., 2022), inhibits lipid peroxidation, thus preventing membrane damage and the modification of low-density lipoproteins. Moreover, vitamin E and vitamin C work together to protect vulnerable polyunsaturated fatty acids such as omega-3s. And, as we shall see later, an omega-3 fatty acid, docosahexaenoic acid (DHA), is a key molecule in GLUT-1 transporter function. In fact, brain tissue is especially sensitive to oxidative injury because of its higher metabolic rate driven by glucose and oxygen (reactive oxygen species are generated continuously during oxidative metabolism), lower concentrations of protective antioxidants, and higher levels of polyunsaturated fatty acids that are susceptible to lipid peroxidation (Markesbery, 1999). The most reactive forms, such as the hydroxyl radical, are also capable of oxidizing proteins and nucleic acids.
(Daskalopoulos et al., 2002). Being the transporter that enables glucose and DHAA to cross the blood-brain barrier, this further reinforces the essential role of GLUT-1. It allows the production of ATP, glutamate (Kreb’s cycle), and glutathione from glutamate and L-ascorbic acid. These molecules are used in neurotransmission, energy production and the synthesis of antioxidants. GLUT-3 also mediates DHAA transport with a lower effect (Rumsey et al., 1997). The release of ascorbate from astrocytes is associated principally with the activity of glutamatergic neurons (Castro et al., 2009).

5.3 Translocation of GLUT-1 transporter from intracellular stores

We will describe the body’s second pathway for increasing the uptake of glucose, as well as DHAA, namely to increase the GLUT-1 density by relocating this transporter from intracellular stores. Concentration changes in ATP and AMP are the main regulators of AMPK activity. AMPK is a key sensor of cellular energy status based on the AMP/ATP ratio. It is activated by AMP allosterically and inhibited by ATP. The two nucleotides compete for the same binding site on the regulatory subunit (2 Bateman domains) (Adams et al., 2004). Then, Thr172 of a subunit catalytic site is phosphorylated by Liver kinase B1 (LKB1). This phosphorylation is essential for the activity of AMPK. AMPK activity has been shown to increase in neuronal tissue in response to glucose deprivation, metabolic stress, hypoxia and ischemia (Culmsee et al., 2001; Gadalla et al., 2004; McCullough et al., 2005). AMPK activation promotes the translocation of GLUT-3 storage vesicles on the surface of neuronal cells (Weisová et al., 2009). Increasing the amount of GLUT-3 promotes the entry of glucose. At the astrocyte, AMPK activation promotes the translocation of GLUT-1 storage vesicles to the blood brain barrier (Cura et al., 2012). This translocation of GLUT-1 is made from a reservoir of cytoplasmic vesicles (Widnell, 1995). AS160 (Akt substrate of 160 kDa), a Rab GTPase-activating protein that is widely expressed in vertebrates, is located on the membranes of these intracellular vesicles. In the absence of phosphorylation, it maintains these vesicles in the cytoplasm and inhibits its translocation. The phosphorylation of AS160 is dependent on PI3K/Akt pathway (Kim et al., 2011). And remember that the MAPK pathway works in synergy with and depends on PI3K/Akt signaling pathway (see mechanism below). After the phosphorylation of AS160 (Treebak et al., 2006), GLUT-1 translocates to and enters the BBB (Andrisse et al., 2013; Fig. 2). The role of AS160 is mediated by its GTPase-activating domain and interactions with Rab proteins in vesicle formation, increasing GLUT-1 translocation when its GTPase activity is inhibited by AMPK phosphorylation. AS160 is not only an Akt substrate but also a substrate for other kinases such as AMPK (Sakamoto et al., 2008). Phosphorylation appears to occur at different sites (Ser or Thr) (Chen et al., 2008). Eight residues on AS160 (Ser318, Ser341, Thr568, Ser570, Ser588, Thr642, Ser666, Ser751) that can be phosphorylated have been identified. AMPK preferentially phosphorylates Ser588, with less phosphorylation of other sites (Geraghty et al., 2007). These actions have been described for GLUT-1 and GLUT-4 (Sakamoto et al., 2008; Marko et al., 2020) in skeletal muscle. It can be assumed to apply to astrocytes and neurons, to GLUT-1 and GLUT-3 (Cura et al., 2012; Fig. 2).

AMPK can also be phosphorylated and activated by the AMPK kinase Ca2+/calmodulin-dependent protein kinase kinase β (CaMKKβ) (Hawley et al., 2005) (Fig. 2). CaMKKβ is a serine/threonine-directed kinase that is activated following increases in intracellular Ca2+ concentrations. The level of glutamatergic activity determines the level of Ca2+ that enters astrocytes and postsynaptic neurons. A key receptor is involved, the NMDA receptor is a gateway for Ca2+. NMDA agonists such as glutamate can cause a rise in intracellular Ca2+ levels within astrocytes (Lee et al., 2010). NMDA receptors are Ca2+-permeable and stimulate Ca2+ influx into the cell. The activation of CaMKKβ facilitates the translocation of GLUT-1 and GLUT-3 as described above. Thus, if the role of AMPK is to promote ATP conservation and production, AMPK does not just react to cellular energy depletion but also anticipates it via extracellular Ca2+ flux. AS160 also contains a calmodulin-binding domain, and this domain mediates phosphorylation (Kramer et al., 2007); the calmodulin-dependent protein kinase II (CaMKII) is involved (Mohankumar et al., 2012). The levels of phosphorylation of AS160 at its multiple phosphorylation sites may be such that AMPK-induced phosphorylation alone would limit its reaction speed. The coupling of the two means of phosphorylation (AMPK and calmodulin) would accelerate the kinetics.

5.4 Lactate and the role of monocarboxylate transporters (MCTs)

The transport of lactate from astrocytes to pre- and postsynaptic neurons by proton-linked monocarboxylate transporters (MCTs) (Halestrap et al., 1999; Pérez-Escuredo et al., 2016) has several advantages: (i) the removal of lactate and its proton avoids acidosis of astrocytic cytosol (low pH inhibits phosphofructokinase activity and slows or stops anaerobic glycolysis), (ii) the catabolism of lactate in neurons, once again via pyruvate. With lactate, neurons can respond faster to stimulation than they can with aerobic glycolysis from glucose. MCTs are found in many types of tissue, including the brain where three isoforms, MCT1, MCT2 and MCT4 (monocarboxylate transporters), have been described. Each of these isoforms exhibits a distinct regional and cellular distribution in rodent brains. At the cellular level, MCT1 is expressed by endothelial cells of microvessels, by ependymocytes, and by astrocytes. MCT4 expression appears to be specific to astrocytes. MCT2 is mainly expressed in neurons (Pierre et al., 2005). Their anchorage and activity at the plasma membrane requires interaction with a chaperone protein, such as basigin (CD147) or embigin (gp70), which belong to the immunoglobulin superfamily. These proteins are anchored to the plasma membrane through a single transmembrane domain containing a conserved glutamate residue. Basigin (Muramatsu et al., 2016) is more widely expressed cell tissue than embigin, and appears more frequently as the preferential partner for MCT1 and MCT4, whereas MCT2 preferentially interacts with embigin (Wilson et al., 2005). Basigin expression (BSG gene) in the mouse uterine epithelium appears to be upregulated by estrogen via the estrogen receptor-α (ERα) (Chen et al., 2010). Embigin expression...
Glucose and lactate provide a complementary supply of energy according to different spatiotemporal modalities. Thus, estradiol has an important role in the transport of lactate by GLUTs, whose mechanism will be detailed in the following sections of the article in addition to the synergistic interaction between estradiol and DHA.

6 Free estradiol as key regulator of regional cerebral glucose uptake

Baseline regional cerebral glucose metabolism differs between males and females (Gur et al., 1995). Neuroimaging studies have demonstrated that both age and sex affect cerebral glucose metabolism (Kim et al., 2009). Ovarian steroids may mediate alterations in glucose uptake because the highest glucose uptake occurs during pro-oestrus (Neblich et al., 1985), and cerebral glucose metabolism decreases in postmenopausal women who are not receiving estrogen replacement therapy (Rasgon et al., 2005). Cerebral blood flow is diminished in hypoestrogenic women, with regional patterns resembling those of patients with mild to moderate Alzheimer’s disease (Greene, 2000). Gender differences exist in the gene expression profiles of GLUT-1 in mouse tissues (Nagai et al., 2014). Hormonal variation across the four stages of the rat oestrus cycle affects the abundance of mRNA of cerebral transporters GLUT-1 and GLUT-3 in the hypothalamus, hippocampus and prefrontal cortex (Harrell et al., 2014).

6.1 Free estradiol and regional cerebral glucose flow regulation by GLUT-1 transporters

17β-estradiol (estradiol) is an estrogen hormone that plays an essential role in the up-regulation of GLUT-1 in the brain. It increases the number of GLUT-1 molecules on the astrocyte and capillary endothelial cell membranes in contact with the blood-brain barrier. Its intervention takes place in at least three stages (synthesis, translocation, and anchoring) to increase the flow of glucose and DHAA in response to stimuli (Majou, 2018).

The SLC2A1 gene, which codes for GLUT-1, is an estrogen-regulated gene with transcription activation by estrogen receptors (ERs) (Wang et al., 2004), which are also present on astrocyte membranes (Chaban et al., 2004). In vivo, treatment with estradiol increases GLUT-1 protein concentration in the blood-brain barrier’s endothelial cells, and GLUT-1 mRNA expression in correlation with the increase in glucose uptake (Shi et al., 1997). The same effect also appears in fetal rat lungs (Hart et al., 1998). This treatment induces a two- to fourfold increase in GLUT-3 mRNA levels and lesser but significant increases in GLUT-3 protein levels, as well as a 70% increase in parenchymal GLUT-1 mRNA levels in the primate cerebral cortex (Cheng et al., 2001).

Once synthesized, GLUT-1 protein is stored in vesicles. As described above, AMPK activation promotes the translocation of GLUT-1 and GLUT-3 to the BBB. And estradiol allows the translocation of GLUT-1 vesicles to the cell membranes, favoring the phosphorylation of a vesicle protein (AS160) via the PI3K/Akt signaling pathway or via activation/phosphorylation of AMPK (Rogers et al., 2009) by the LKB1 enzyme, coded by the STK11 gene (Serine-Threonine Kinase 11). Estradiol also increases the expression of this STK11 gene, an effect mediated by ERα (Mac Innes et al., 2012). Lastly, the GLUT-1C terminus is anchored to the sub membranous actin cytoskeleton by a scaffolding protein known as GIPC1 (GAIP-interacting protein, C terminus) or GLUT-1CBP (GLUT-1 C-terminal binding protein) (Hernandez-Garzón et al., 2016; Reed et al., 2005). Estradiol increases the expression of GIPC1 mRNA (RGD). The highest amount of GIPC1 mRNA is observed in the brain (Bunn et al., 1999). Blocking GLUT-1 interactions with GIPC1 disrupts normal GLUT-1 trafficking, leading to a reduction in the level of GLUT-1 in the plasma membrane and concomitant accumulation in internal membrane structures (Reed et al., 2005). A GIPC1 deficiency decreases GLUT-1 surface levels and glucose uptake (Wieman et al., 2009).

Thus, free estradiol — without its binding protein (sex hormone binding globulin, SHBG) — plays a significant role in the up-regulation of GLUT-1 during its synthesis by the expression of the SLC2A1 gene, as well as translocation by activating AMPK via the LKB1 gene and its anchor GIPC1 to increase the number of GLUT-1 molecules in astrocytes and capillary endothelial cells (Fig. 2). Therefore, if the concentration of free estradiol falls, there is competition between all estrogen receptors and SHBG (see below) and uses thereof will take place with a consequence on GLUT-1 activity kinetics (number of operational GLUT-1 per unit of time). This is demonstrated by the delayed response to cellular demand for glucose and DHAA, resulting in energy and antioxidant imbalance (FEDOX paradigm). If astrocytes are relatively well protected, the impact will be on pre- and postsynaptic neurons.

6.2 Free estradiol and free IGF-1: a synergistic effect

Free estradiol has a synergistic effect with free insulin-like growth factor-1 (IGF-1) — without its binding protein (insulin-like growth factor-binding proteins, IGF-BPs) — which in humans is encoded by the IGF-1 gene (Nelson et al., 2014). For example, several studies have shown this interaction in different regions of the brain (Garcia-Segura et al., 2006; Varea et al., 2010; Park et al., 2014; Huffman et al., 2017) and in breast cancer cells (Song et al., 2010). IGF-1 mRNA levels were significantly increased in primate cerebral cortical neurons treated with estradiol (Cheng et al., 2001) and in the immortalized rat hippocampal cell H19-7. This increase in the number of copies of IGF-1 mRNA was accompanied by an increase in IGF-1 protein level (Shingo et al., 2003). In uterus, this IGF-1 synthesis requires ERα, which binds directly to target DNA sequences (estrogen-responsive elements) (Hewitt et al., 2010). IGF-1 is a protein that provides good neuroprotective effects. IGF-1 is produced by the liver as an endocrine hormone as well as in target tissues in a paracrine or autocrine fashion. IGF-1 is synthesized de novo or transported across the BBB. Local production of IGF-1 is believed to be the primary source for brain cells (Russo et al., 2005). IGF-1 is particularly expressed in astrocytes (Chernausek, 1993; Madathil et al., 2013). IGF-1 crosses the BBB from plasma via a saturable transport system.
In serum, only a small amount of IGF-1 circulates free. IGF-1 is sequestered into ternary complexes consisting of one molecule each of IGF-1, IGF binding protein-3 (IGFBP-3), and acid-labile subunit (ALS) (Nishijima et al., 2010). The cleavage of IGFBP-3 by matrix metalloproteinase-9 (MMP9) allows the passage of serum IGF-1 through an interaction with the endothelial transporter lipoprotein related receptor 1 (LRP1) which is abundantly expressed in brain endothelium (Nishijima et al., 2010). PPARγ-RXRα induces the expression of the LRP1 gene (see mechanism below) (Wang et al., 2016).

The anabolic actions of IGF-1 are mediated by the IGF-1 receptors (IGF-1Rs). When IGF-1 binds to the IGF-1R, it causes a conformational change to the receptor, inducing the autophosphorylation of tyrosine residues (Hubbard et al., 2000). This leads to the recruitment of insulin receptor substrates (IRS-1 to IRS-4), which in turn phosphorylates the tyrosine residues of the IRSs. ERα regulates the IGF-1 signaling pathways (Kahlelert et al., 2000) through phosphorylation of ERK1/2 (extracellular signal-regulated kinases) and Akt, and the between ER and IGF-1R potentiates cell growth. Estradiol and IGF-1 stimulate translocation of ERs from the nucleus to the cytoplasm (Song et al., 2010). Estradiol stimulates the rapid activation of the IGF-1Rs through phosphorylation via ERα and induces the formation of a ternary protein complex comprised of phosphorylated Shc protein, ERα and IGF-1R (Song et al., 2004). Adapter protein She generally acts by activating MAPK and PI3K/Akt pathways (Gu et al., 2000; Vindis et al., 2003). The tyrosine phosphorylation of Shc is mediated by the tyrosine kinase Fyn (Src family kinase) associated with caveolin-1 (scaffold-protein) (Wary et al., 1998). Upon integrin ligation, Fyn is activated and binds to Shc via its SH3 domain. She is subsequently phosphorylated at tyrosine 317 (Wary et al., 1998). The activation of IGF-1Rs stimulates MAPK kinase and, consequently, the phosphorylation of ERK1/2. The activation of ERK1/2 may in turn lead to the phosphorylation of ERα (Kato et al., 1995) (Russo et al., 2002). Moreover, She is expressed in astrocytes (Cazaubon et al., 1994). The activation of membrane ERα appears to be coupled to the MAPK pathway (Pawlak et al., 2005). This process leads to the activation of two main downstream signaling pathways: PI3K/Akt and MAPK cascades (Zheng et al., 2000). The interaction of the ER and IGF-1R is important for the non-genomic effects of ER. (Yu et al., 2013; Fig. 2). It is also worth noting that the IGF-1R is capable of heteromeric assembly with the insulin receptor (IR). Heterodimers exhibit a similar affinity to IGF1 as IGF-1R homodimers but have a substantially lower insulin binding affinity (Bailyes et al., 1997; Kleinridders, 2016), indicating that the action of IGF-1 is crucial for brain physiology. Indeed, IGF-1 is at least 10 times more potent than insulin in stimulating the rate of glucose uptake, showing that IGF-1, rather than insulin, is the physiological agonist regulating glucose transport in ependymal cells (Verleysdonk et al., 2004). Insulin appears to stimulate brain glucose metabolism at physiological postprandial levels in patients with impaired glucose tolerance (diabetes), but not in healthy subjects (Hirvonen et al., 2011), perhaps through glucagon-like peptide-1 (GLP-1) system (Sandoval and Sisley, 2015).

6.3 Estradiol and regional cerebral glucose flow regulation by nitric oxide (NO)

The phosphorylation of eNOS resembles that of AS160 via the activation/phosphorylation of AMPK or via the MAPK and PI3K/Akt pathways (Fig. 2). In the LKB1/AMPK/eNOS pathway, estradiol induces transcription of the LKB1 gene (Mac Innes et al., 2012). The second pathway is a non-genomic interactive effect of free estradiol and free IGF-1, which rapidly phosphorylates eNOS via the PI3K/Akt dependent pathway (Dimmel et al., 1999). This activation is mediated by ERα (Gerhard et al., 1996) localized to caveolae in endothelial cells lining cerebral capillaries (Isenovic et al., 2003; Stirone et al., 2005; Chambless et al., 2005). The process also requires phosphorylated Shc and Fyn (Src kinase) associated with caveolin-1 (Kim and Formoso, 2007). Moreover, Src kinase mediates ERβ-induced increases in phosphorylation levels of nNOS at S1412 and NO production by activating the PI3K/Akt pathway (Gingerich et al., 2008; Fig. 2).

An increase in NO is also due to increases in eNOS and nNOS expression. The effects on eNOS and nNOS abundance are primarily mediated at the level of gene transcription. Estradiol is known to induce an upregulation of eNOS gene expression, through the binding of ERα to its eNOS promoter through the estrogen-responsive element (Min, 2007). Endogenous variations in estrogen levels during the estrous cycle also coincide with corresponding changes in the state of nNOS Ser1412 phosphorylation (Parkash et al., 2010). Estradiol increases nNOS and eNOS expression and activity in the female hippocampus and thus improves hippocampal function (Grohe et al., 2004). Both pregnancy and estradiol treatment increase the amount of NOS isozyme, eNOS and nNOS mRNA in skeletal muscle (Weiner et al., 1994). Overall, estradiol stimulates the NO neuronal system. Reduced eNOS and nNOS expression have been associated with stroke and Alzheimer’s disease, which is associated with neurovascular dysfunction (Thorns et al., 1998; Tan et al., 2015).

6.4 Astrocytes: endogenous and exogenous sources of estradiol

The supply of 17β-estradiol can have several origins depending on the quantities to be supplied and local consumption. As we will see, it will depend on the plasma concentrations of estradiol and testosterone, and their modulator, SHBG, at every stage of life (puberty, elderly, menstrual or estrous cycle, pregnancy), physiological status, or exogenous hormones (contraceptive pills, phytoestrogens, etc.). Thus, the action of estradiol is regarded as endocrine, autocrine and paracrine.

Estradiol may have three sources, including an exogenous source, directly from plasma through gonadal synthesis; the free form of estradiol crosses the BBB thanks to its lipid-permeable nature by a passive diffusion mechanism (Partridge et al., 1979). But the concentration of estrogens circulating in the blood may be much lower than that of local intra-tissue estrogen production, particularly in men and postmenopausal women (Simpson et al., 2005). And a first local endogenous source, from testosterone from the plasma through gonadal synthesis. The free form of testosterone also crosses the BBB.
thanks to its lipid-permeable nature. Testosterone is the precursor of estradiol by aromatization. The P450 aromatase (CYP19 gene) is a microsomal enzyme of the cytochrome P450 family (Roselli et al., 2009). Neurons, astrocytes and brain capillary endothelial cells express P450 aromatase. In astrocytes, estradiol concentrations are proportional to the activity and expression of aromatase (Saleh et al., 2005).

The second local endogenous source is cholesterol, which is synthesized from acetyl-CoA, which may partly be produced from glucose and from fatty acids. The brain is a steroidogenic organ that expresses steroidogenic enzymes and produces neurosteroids. In studies on rat brains (Zwain and Yen 1999), astrocytes appear to be the most active steroidogenic cells. They express a cholesterol side-chain cleavage enzyme (P450scct) (mitochondrial enzyme), 17α-hydroxylase/C17,20 lyase (P450c17), 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD), and P450 aromatase and produce pregnenolone, progesterone, dehydroepiandrosterone (DHEA), androstenedione, testosterone, estradiol, and estrone.

A rate-limiting step in neurosteroid synthesis from cholesterol is the transport of cholesterol mediated by apolipoprotein E (ApoE). ApoE is expressed in such high concentrations that the brain is the organ with the second highest ApoE expression after the liver. ApoE is expressed within the brain predominantly by astrocytes, oligodendrocytes, microglia and epidermal layer cells (Orth et al., 2012) while neurons preferentially express the receptors for ApoE. ApoE does not cross the BBB and, therefore, must be synthesized locally in the brain (Liu et al., 2012). This protein is undoubtedly involved in brain cholesterol homeostasis. It transports cholesterol to neurons via ApoE receptors. Free 17β-estradiol regulates ApoE gene expression in a tissue-specific manner in mice, in particular free estrogen increases ApoE mRNA and protein expression in the brain (Srivastava et al., 1996; Stone et al., 1997) via the activation of ERα (Struble et al., 2003; Wang et al., 2006).

The other rate-limiting step in neurosteroid synthesis from cholesterol is the transport of cholesterol into the mitochondria. Steroidogenic acute regulatory protein (StAR) is the protein involved in this transport of cholesterol (Stocco, 2001). StAR transcripts are abundant in the cerebral cortex, hippocampus, dentate gyrus, olfactory bulb, cerebellar granular layer, and Purkinje cells. In addition its cellular distribution overlaps with that of P450scct and 3β-HSD (Furukawa et al., 1998). These findings implicate StAR in the biosynthesis of neurosteroids. It is important to note that the expression of PPARγ-RXRα increases StAR-promoter activity in KK1 mouse granulosa cells and MA-10 mouse Leydig tumor cells (Kowalewski et al., 2009), and DHA is a preferential ligand compared to PPARs and RXRs (de Urquiza et al., 2000; Diep et al., 2002; Deckelbaum et al., 2006; Song et al., 2017; Dziedzic et al., 2018).

6.5 By binding to PPARγ-RXRα, DHA therefore blocks transcription of SHBG gene and increases the quantity of free estradiol

However, the biologically active fraction of estradiol is its non-protein-bound, free fraction (Hammond, 2016). The role of binding proteins is essential in the modulation of the distribution of active molecules in space and over time, and in their protection against lysis. The regulation is dependent on the strength of the molecular bond; low affinity causes rapid action while high affinity allows a more progressive distribution compared to the “all or nothing” action of synthesis. Albumin and sex hormone-binding globulin (SHBG) modulate the availability of estradiol. SHBG, an extracellular glycoprotein, is produced mostly by the liver and is released into the bloodstream. Other sites that locally produce SHBG include the brain (Herbert et al., 2005), uterus, testes and placenta. SHBG concentrations vary with age (Harman et al., 2001; Maggio et al., 2008). In adults, SHBG is on average twice as high in women than in men (Carlström et al., 1990). Testosterone lowers SHBG levels, while inversely estradiol raises them (Carlström et al., 1990; Cunningham et al., 1984). For example, SHBG concentrations rise significantly under oral contraceptive treatment (Panzner et al., 2006), as well as during pregnancy. Albumin has low affinity and high capacity for estradiol, contrary to SHBG which has high affinity and low capacity (stable binding constant for albumin: 4.21 × 10⁸ L/mol, and for SHBG: 3.14 × 10⁸ L/mol) (Södergard et al., 1982; Cunningham et al., 1984). All of the above are in a dynamic, competitive equilibrium. The "bioavailable fraction" is the sum of the "free fraction" and the albumin-bound fraction. The SHBG-bound fraction of estradiol is not biologically active. Thus, the SHBG concentration is decisive for the bioavailability of estradiol and its activity. The promoter region of the human SHBG gene contains PPRE. PPARy-RXRα represses the expression of SHBG in liver cells, while different PPARy-RXRα levels and activity contribute directly to the variations in plasma SHBG levels (Selva et al., 2009). DHA is a preferential ligand in comparison to PPARs and RXRs. DHA increases the activity of PPARy (Hwang et al., 2017; Naeini et al., 2020; Song et al., 2017). By binding to PPARy-RXRα, DHA therefore blocks transcription of SHBG gene, reduces the concentration of SHBG, and increases the quantity of free estradiol. This favors the activity of GLUT-1 and the flow of glucose and DHA.

7 DHA as key regulator of regional cerebral glucose uptake

7.1 Gene transcription modulation by DHA

DHA also plays an essential role as a gene transcription modulator via transcription factors, in particular peroxisome proliferator activated receptors (PPARs) and retinoid X receptors (RXRs). These transcription factors take the form of PPAR-RXR heterodimers, located within the nucleus and activated by phosphorylation and their respective ligands, which modify their tertiary structures and enable them to bind to the PPRE located in the promoter region of target genes. In the absence of ligands, PPARs form complexes with corepressors such as NCoR, RIP140 or SMRT, which repress transcription through the recruitment of histone deacetylases. In the presence of ligands, coactivators such as p300, CBP or SRC-1 become bound to the amino terminal of PPARs (Moreno et al., 2010). As we will see later, the heterodimer bond on the PPRE activates the transcription of genes such as FADS2 (Majou, 2021), LRPI (Wang et al., 2016), StAR...
and RXRs (de Urquiza et al., 2009) represses the expression of genes such as SHBG (Selva et al., 2009). DHA is a preferential ligand in comparison to PPARs and RXRs (de Urquiza et al., 2000; Diep et al., 2002; Deckelbaum et al., 2006; Song et al., 2017; Dziedzic et al., 2018).

7.2 Up-regulation of eNOS and nNOS by DHA

The consumption of fish or fish oil influences the expression of various vasoactive molecules and NO production by nNOS (Engström et al., 2009) and eNOS gene expression in endothelial cells. Furthermore, DHA induces endothelium-dependent NO-mediated relaxation in the coronary artery. In cultured human coronary artery endothelial cells, DHA enhanced NO production and the activity of eNOS. In addition, it enhanced the expression of eNOS and phospho-eNOS. Specifically, DHA stimulated eNOS and PI3K/Akt activity, and induced NO bioavailability in response to Akt kinase activation (Stebbins et al., 2008). eNOS activity in endothelial cells is modulated by DHA via MAPK pathway (Huang et al., 2021; Fig. 2). p38 MAPK regulates both the activity and expression of eNOS by DHA (Huang et al., 2023). PPARα and PPARγ induce activation of members of the MAPK family (p38 MAPK) (Gardner et al., 2003). From all these converging experimental results, there is evidence that DHA, a preferential ligand for PPARs and RXRs, modulates eNOS activity via p38 MAPK. DHA treatment protects dopaminergic neurons in substantia nigra increasing nNOS in the experimental mice model of Parkinson’s disease (Parlak et al., 2018). So, it is plausible that the mechanisms are identical for nNOS.

7.3 Up-regulation of GLUT-1 transporters by DHA

DHA plays a major role in the up-regulation of GLUT-1. For example, in elderly monkeys, supply of DHA results in a significant increase in regional cerebral blood flow response to stimulation (Tsukada et al., 2000). In humans, quantitative erythrocyte EPA/DHA levels are related to a higher regional cerebral blood flow in the brain (Amen et al., 2017). N-3 PUFA deficiency specifically decreases the GLUT-1 protein content of both endothelial cells and astrocytes in rats (Pifferi et al., 2005; Harbeby et al., 2012). Lower levels of GLUT-1 transporters result from a reduction of the transcription of the SLC2A1 gene encoding the two GLUT-1 isoforms (Harbeby et al., 2012). This reduction in gene expression was also found during neuronal activation, supporting the hypothesis that the alteration of glucose uptake due to n-3 PUFA deficiency persists during brain activation (Harbeby et al., 2012). Long-chain n-3 polyunsaturated fatty acid supplementation (mainly EPA and DHA) improves brain glucose uptake and metabolism in adult non-human primates (Pifferi et al., 2015; Harbeby et al., 2012). In primate studies, DHA levels are proportional to local cerebral metabolic rate of glucose uptake. This is correlated to a higher DHA concentration in cells and tissues, which associated with high energy consumption, consistent with high DHA levels in mitochondria and synaptosomes (Brenna et al., 2007). In addition, the activity of the Na⁺/K⁺-ATPase pump is severely reduced in nerve endings in the whole brain of n-3 PUFA-deficient rats (Bourre et al., 1989). These results confirm that physiological doses of DHA have a direct and positive effect on glucose transport and density of the two isoforms of GLUT1 (endothelial and astrocytic) (Pifferi et al., 2010).

On the other hand, the specificity of the n-3 effect is highlighted by the absence of an effect on neuronal GLUT-3 glucose transporters. The expression of neuronal GLUT-3 does not change (Pifferi et al., 2005), supporting the concept that neurons are metabolically unable to increase their glucose uptake and utilization upon activation to sustain ATP demand (Choeiri et al., 2005).

7.4 Origin and synthesis of DHA in the brain

Mammals are incapable of synthesizing α-linolenic acid, the precursor of the omega-3 polyunsaturated fatty acids, de novo. They are totally dependent on the intake of dietary precursors. The supply of DHA to the brain is thought to be governed by a principle of energy optimization. The brain is capable of autonomous DHA synthesis from α-linolenic acid (ALA, 18:3 n-3), the essential precursor for DHA. However, it prefers an exogenous source of DHA from the blood, via the blood-brain barrier. This supply can come from four different, non-exclusive sources, depending on the concentrations of supply and consumption. Two sources are exogenous to the brain, from the blood: (i) directly from the diet in via the transport of lysophosphatidylcholine-DHA across the BBB most likely occurs through major facilitator superfamily domain-containing protein 2 (MFSD2) – a membrane transport protein that is expressed in the endothelium of the BBB (Nguyen et al., 2014; Thies et al., 1994) (ii) by synthesis in the liver from dietary ALA, the essential precursor for DHA - but less than 5% ALA is converted into DHA. This rate depends on the concentration of n-6 fatty acids and long chain polyunsaturated fatty acids in the diet (Brenna, 2002). The two other sources are: (i) from membrane phospholipids, which are a major component of all nerve cell membranes (Jump, 2002); the enzymes responsible for its release are intracellular phospholipases of the A2 family as calcium-independent phospholipase A2 (iPLA2) that releases docosahexaenoic acid (DHA) from glycerophospholipids (Capper et al., 2001; Rosa et al., 1791) (ii) by synthesis in the astrocytes from dietary ALA via the blood-brain barrier. Neurons do not have the desaturases required to synthesize these omega-3 lipids (DHA). Astrocytes, on the other hand, elongate and desaturate the 18- and 20-carbon precursors. The majority of the long-chain fatty acids formed by astrocytes, particularly DHA, are released into the extracellular fluid for uptake by neurons (Moore et al., 1991). The relationship between diet, blood synthesis and astrocyte synthesis are still a subject of debate (Bewicz-Binkowska et al., 2019). A more efficient route of incorporation of DHA into brain lipids is via DHA itself derived from food or phospholipids or by metabolism in the liver, rather than by metabolism from ALA in astrocytes (Sinclair et al., 1972). However, when DHA levels in the brain decline, a secondary synthesis appears to take place in astrocytes in an attempt to offset dietary deficiencies and ensure neuronal DHA homeostasis (McNamara et al., 2008).
The DHA synthesis pathway is known as Sprecher’s shunt. From ALA found in the diet, a series of enzyme transformations, including two desaturases (Δ6-desaturase and Δ5-desaturase) and elongases in the endoplasmic reticulum, followed by peroxisomal β-oxidation, results in DHA (Voss et al., 1991). Δ6-desaturase catalyzes two essential stages of DHA biosynthesis (Cho et al., 1999; Stoffel et al., 2008). As the second stage of desaturation by this enzyme is limiting, it makes Δ6-desaturase a key enzyme in DHA synthesis (Lattka et al., 2010; Tosi et al., 2014; O’Neill et al., 2017; Delplanque, 2017). The amount of DHA produced is directly linked to the level of Δ6-desaturase available, but also to the ALA content. The rate of conversion from ALA to DHA depends on the type of tissue. Astrocytes are believed to have exceptional productivity (Barceló-Coblijn et al., 2005). The ratio of omega-3/omega-6 fatty acids is also important for efficient conversion. Non-converted ALA undergoes aerobic β-oxidation in astrocyte mitochondria (Edmond et al., 1987) and is fully degraded into acetyl-CoA (Lynen’s helix). In humans, the FADS2 gene (Δ6-desaturase gene) is ubiquitously expressed, especially in the liver and brain (astrocytes) (Innis et al., 2002; Nakamura et al., 2004), as well as in the heart, skeletal muscle, kidney, lung, prostate, testes, adipocytes, ovary, uterus and sebaceous glands (Ge et al., 2003; Nwankwo et al., 2003; Pédrono et al., 2010). The phosphorylated PPARα-RXRα heterodimer modulates the transcription of the FADS2 gene (Tang et al., 2003; Majou, 2021). Free estradiol induces the activation of PPARα via two pathways. The first, by transcription, through its genomic action on the PPARα gene, which is mediated by an estrogen receptor (Campbell et al., 2003). The second involves a non-genomic effect which, through phosphorylation exclusively on serine residues, increases the transcriptional activity of PPARα, via the ERK1/2-MAPK pathway (Majou, 2021) with a strong functional cooperation with PGC-1, a known ligand-influenced PPARα coactivator (Barger et al., 2001). The expression of Δ6-desaturase is retro-inhibited by intracellular free DHA (Matsusaka et al., 2002; Gibson et al., 2013; Bewicz-Binkowska et al., 2019; Majou, 2021).

8. What are the relationships between the up-regulation of regional cerebral glucose uptake by free estradiol and by DHA?

There are close molecular relationships between the up-regulation of the GLUT-1 and NO pathways by free estradiol and by DHA. First, the interactions between DHA and estradiol occur in particular via the phosphorylated PPARs-RXR heterodimers, one of whose preferential ligands is DHA (de Urquiza et al., 2000; Diep et al., 2002; Deckelbaum et al., 2006; Song et al., 2017; Dziedzic et al., 2018). As described above, PPARγ-RXRα blocks the transcription of the SHBG gene, reduces the concentration of SHBG, and increases the quantity of free estradiol, and consequently DHA boosts the action of estradiol.

PPARα-RXRα and PPARγ-RXRα, with their ligand DHA, induce activation of members of the MAPK family (Gardner et al., 2003) which activate the IGF-1/Estradiol/PI3K/Akt signaling pathway.

And, as we have already described, the PPARα-RXRo heterodimer modulates the transcription of the FADS2 gene (Tang et al., 2003). Free estradiol induces the activation of PPARα via two pathways: (i) transcription, through its genomic action on the PPARα gene, which is mediated by an estrogen receptor; (ii) a non-genomic effect that allows for phosphorylation and activates PPARα via the ERK1/2-MAPK pathway (Majou, 2021). As confirmation of this mechanism, observational evidence suggests that in populations that consume low levels of n-3 highly unsaturated fatty acids, women have higher blood DHA levels than men (Kitson et al., 2010). Women of reproductive age are known to convert more ALA into DHA than men (Burdge et al., 2002; Magnusardottir et al., 2009). Estrogens cause higher DHA concentrations in plasma cholesteryl esters in women than in men by up-regulating synthesis from ALA. This difference is independent of dietary differences. Moreover, it has also been suggested that estradiol may increase the activity of the desaturation pathway because DHA synthesis is shown to be almost three times greater in women who use an oral contraceptive pill that contains 17-ethinylestradiol than in women who do not, whereas a testosterone stimulus induces a decrease in DHA status (Giltay et al., 2004). This difference in conversion appears to be associated with estrogen and some evidence indicates that the expression of enzymes, including desaturases, involved in synthesizing DHA from ALA is higher in females (Kitson et al., 2013).

9 Conclusion

During neuronal activity, glucose metabolism and cerebral blood flow are closely coordinated to maintain proper brain function. The uptake of glucose and DHAA across the BBB is facilitated by a single carrier protein, the GLUT-1 transporter. Then, lactate transport between astrocytes and neurons is achieved by MCT transporters. Cerebral blood flow is regulated by the nitric oxide pathway. All steps of the process are complementary and highly controlled in order to guarantee the kinetics at the synapses, according to the neuronal stimulations, and thus glutamate metabolism, neurotransmission and cell viability.

A tandem of two key molecules, free estradiol and DHA is involved in this critical regulation. Their relationship is synergistic and reciprocal: free estradiol with genomic and non-genomic actions via ERα, and DHA via the PPARα-RXRα and PPARγ-RXRα heterodimers. From a certain level of chronic DHA and free estradiol deficiency, a permanent imbalance is established with a disruption of glucose intake and cerebral metabolism. This depletion, particularly in DHA, is associated with pathologies of the central nervous system encompassing neurodegeneration and cognitive defects. It is an aggravating factor in certain pediatric neuropathologies such as hyperactivity, learning difficulties (Milte et al., 2012), mental retardation (Neggers et al., 2009), epilepsy (Emory University Health Sciences Center, 2004) and autism (Bent et al., 2009; Sun et al., 2018). In the elderly, DHA depletion is an aggravating factor in the etiology of Alzheimer’s disease (Majou, 2015), for example. The origins of this deficiency are multiple: (i) a genetic polymorphism that has an impact on various proteins (transporters, enzymes, etc.), in particular the
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