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Brain energy metabolism spurns fatty acids as fuel due to their inherent mitotoxicity and potential capacity to unleash neurodegeneration

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ABSTRACT

The brain uses long-chain fatty acids (LCFAs) to a negligible extent as fuel for the mitochondrial energy generation, in contrast to other tissues that also demand high energy. Besides this generally accepted view, some studies using cultured neural cells or whole brain indicate a moderately active mitochondrial β-oxidation. Here, we corroborate the conclusion that brain mitochondria are unable to oxidize fatty acids. In contrast, the combustion of liver-derived ketone bodies by neural cells is long-known. Furthermore, new insights indicate the use of odd-numbered medium-chain fatty acids as valuable source for maintaining the level of intermediates of the citric acid cycle in brain mitochondria. Non-esterified LCFAs or their activated forms exert a large variety of harmful side-effects on mitochondria, such as enhancing the mitochondrial ROS generation in distinct steps of the β-oxidation and therefore potentially increasing oxidative stress. Hence, the question arises: Why do in brain energy metabolism mitochondria selectively spurn LCFAs as energy source? The most likely answer are the relatively higher content of peroxidation-sensitive polyunsaturated fatty acids and the low antioxidative defense in brain tissue. There are two remarkable peroxisomal defects, one relating to α-oxidation of phytanic acid and the other to uptake of very long-chain fatty acids (VLCFAs) which lead to pathologically high tissue levels of such fatty acids. Both, the accumulation of phytanic acid and that of VLCFAs give an enlightening insight into harmful activities of fatty acids on neural cells, which possibly explain why evolution has prevented brain mitochondria from the equipment with significant β-oxidation enzymatic capacity.

Contents

Abbreviations: AcAc, acetoacetate; BBB, blood-brain barrier; CRC, Ca^{2+} retention capacity; ETF, electron transfer flavoprotein; IMM, inner mitochondrial membrane; LCFAs, long-chain fatty acids; LCHAD, long-chain 3-hydroxyacyl-CoA dehydrogenase; MCFAs, medium-chain fatty acids; $\Delta\psi_m$, mitochondrial membrane potential; MTP, mitochondrial trifunctional protein; PUFAs, polyunsaturated fatty acids; PPAR, peroxisome proliterator activator receptor; RBM, rat brain mitochondria; RLM, rat liver mitochondria; ROS, reactive oxygen species; βOHB, ß-hydroxybutyrate; VLCFAs, very long-chain fatty acids; X-ALD, X-linked adrenoleukodystrophy.

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1. Introduction: brain lipids and cerebral energy metabolism at a glance

Brain consists mostly of fat. Therefore, already Stone Age people used animal brains for tanning of hides. Brain fat contains a high variety of complex lipids, which are characterized by a unique composition of polyunsaturated fatty acids (PUFAs), dominated by arachidonic and docosahexaenoic acid (Brenna and Diau, 2007; Brenna and Carlson, 2014; Chen and Bazinet, 2015). Docosahexaenoic acid plays a particular role within the brain, mostly due to its involvement in the neuron-to-neuron communication (Cunnane and Crawford, 2014). It is also crucial that the abundance of cerebral fatty acids is modulated by the diet (Horwitt et al., 1959; Brenna and Carlson, 2014). Moreover, the content of major lipids, such as phosphatidylcholine and sphingomyelin, alters in an age-dependent manner (Dawson, 2015). Some lipids operate at low quantities in signaling pathways.

From the bioenergetic point of view it is important to emphasize that brain is a high oxygen consumer, accounting in the newborn human brain for up to 74% and in the adult brain to about 20–23% of the body's daily energy intake (Cunnane and Crawford, 2014). Several features, such as high oxygen consumption, a large content of peroxidation-sensitive PUFAs and a strong dependency on the supply of glucose are features that make the brain vulnerable to even small metabolic changes. In this review, we discuss the contribution of the β-oxidation of medium- and long-chain fatty acids to the cerebral energy metabolism. Moreover, toxic activities of fatty acids are considered in this context. We take Refsum disease and X-linked adrenoleukodystrophy (X-ALD) to illustrate the pathobiochemical consequences of accumulation of specific fatty acids in the neural tissue.

In the mammalian brain, energy is used mostly for neurotransmission plus maintenance of excitability, intracellular signaling, axonal or dendritic transport, synthesis of neurotransmitters, and to a lower extent protein synthesis (see for review (Ames, 2000) and citations therein). More than 90% of the ATP is generated in mitochondria using oxidative phosphorylation. The largest portion of the ATP turnover occurs in the grey matter, probably during presynaptic and postsynaptic signaling (Clarke and Sokoloff, 1994; Attwell and Laughlin, 2001). Consequently, neurons demand for most of the energy, whereas the energy consumption of astrocytes amounts to only about 5–15% of the total energy requirement of the brain (Clarke and Sokoloff, 1994). The energy metabolism of neurons is mainly aerobic respiration and that of astrocytes mainly anaerobic glycolysis.

In addition, histochemical staining for lactate dehydrogenase and cytochrome *c* oxidase in regions of poor or rich capillary densities indicates that glycolytic and oxidative ATP generation in brain are frequently segregated anatomically (Ames, 2000). Furthermore, in active neural tissue the ATP production cannot rely on sufficient oxygen supply (Ames, 2000; Andres et al., 2008). The high energy required for brain tissue is supplied by multiple interactions between neurons, astrocytes and cerebral blood vessels which guarantees the supply with enough oxygen and oxidizable substrates (recent reviews (Bélanger et al., 2011; Shetty et al., 2012).

Glucose is the main energy substrate for neurons and glia cells (Hu and Wilson, 1997; Dienel, 2012). Generally, energy reserves are low in brain tissue (Ames, 2000) and are limited to a small amount of glycogen (about 3–12 μmol/g tissue), which is exclusively stored in astrocytes (Dienel, 2012; Pellerin and Magistretti, 2012). Glucose liberated from glycogen is degraded by the astrocytic glycolysis and the resulting lactate is supplied to neurons (Hu and Wilson, 1997; Dienel, 2012). This fact uncovers an intensive metabolic cooperation between astrocytes and neurons, and neuronal synaptic activity stimulates the glycolysis in astrocytes (Ames, 2000; Bélanger et al., 2011; Dienel, 2012).

Moreover, liver-derived ß-hydroxybutyrate (βOHB) and acetoacetate (AcAc) are used as fuel by neurons and glia cells extensively during suckling, maturation in young age and upon prolonged fasting (see for reviews (Veech, 2004; Morris, 2005; Cunnane and Crawford, 2014; Achanta and Rae, 2017) and references therein). The passage of βOHB across the blood-brain barrier (BBB) as well as its cellular uptake by neural cells is achieved by members of the monocarboxylate transporters (MCT1,2,4). It is of interest that the energy content of βOHB ($\Delta H^\circ = -243.6$ kcal/mol C_2 units) is higher than that of pyruvate (ΔH° = -185.7 kcal/mol C_2 units) (Veech, 2004), thus explaining that ketone bodies can mostly replace glucose as cerebral high energy fuel (up to 60%). During a long-lasting decline of blood glucose the ketone bodies function as security brain fuel (Cunnane and Crawford, 2014).

In contrast to other organs with high energy demand (heart, liver, kidney), the hydrogen-rich fatty acids are only poorly used in the brain for ATP generation [(Clarke and Sokoloff, 1994; Attwell and Laughlin, 2001; Speijer, 2011; Schönfeld and Reiser, 2013, 2017). Probable reasons for the low utilization of fatty acids as fuel in the brain are discussed in part 2.2 of this review.

2. Fatty acids in brain energy metabolism in health

2.1. Mitochondrial β-oxidation revisited

According to the commonly held view, mammalian brain does not substantially use long-chain fatty acids (LCFAs, C14:0 – C18:0) as fuel in the energy metabolism (for reviews see (Clarke and Sokoloff, 1994; Attwell and Laughlin, 2001; Speijer, 2011). The only exception known so far is that it has been claimed that cultured astrocytes from the developing rat brain (Edmond et al., 1987) and specialized mammalian hypothalamic neurons are able to oxidize fatty acids (McFadden et al., 2014; Byrne et al., 2015).

Nevertheless, several recent reports propose that to some degree mitochondrial β-oxidation takes place in brain tissue (Chen et al., 2014; Panov et al., 2014; Sayre et al., 2017). Firstly, the genetic knock-down of the carnitine palmitoyltransferase-2 in the fruit fly *Drosophila* results in a triglyceride accumulation in the adult brain, a process that is absent in wild-type fruit flies (Schulz et al., 2015). This finding has been taken as indication for the claim that the adult brain catabolizes LCFAs for energy production. Secondly, an active β-oxidation has been postulated from the effect of the carnitine palmitoyl transferase-1 inhibitor methyl palmoxirate on reactive oxygen species (ROS) generation in brain. Since the systemic application of palmoxirate to rats decreased the level of peroxidation products from PUFAs in brain tissue, it has been hypothesized that methyl palmoxirate-linked inhibition of fatty acid degradation prevents β-oxidation-associated ROS generation (Chen

et al., 2014). However, it should be noted that these types of inhibitors have side-effects on mitochondria (Pike et al., 2011), a fact which might modulate the respiratory chain-dependent ROS generation and therefore affect the yield of peroxidation products. Moreover, astrocytes were supplied with [^{3}H]palmitic acid, and its oxidative degradation was estimated from the formation of [^{3}H]H_2O (Sayre et al., 2017). Thereby it was found that the generation of [^{3}H]H_2O declined when the carnitine-dependent uptake of [^{3}H]palmitic acid was blocked.

Curiously, results on fatty acid degradation by astrocytes measured either via liberation of labeled CO_2 (Edmond et al., 1987) or generation of [^{3}H]H_2O (Sayre et al., 2017) contrast with those data, where mitochondrial β-oxidation was estimated from the formation of βOHB (Thevenet et al., 2016). Thus, it was reported that octanoic acid stimulates the formation of βOHB, but not decanoic acid. Such dependency of βOHB generation on the type of fatty acids seems most unlikely, since previous work showed that the astrocytes-promoted ketogenesis is similar with the usage of octanoic acid and palmitic acid (Auestad et al., 1991). Further confusion arises from the finding that the octanoic acid-stimulated ketogenesis of astrocytes (indicative for β-oxidation), was paralleled by a lowering of the ratio of NAD(P)H to NAD(P^+) (indicative for decreased hydrogen supply) and a reduction of the mitochondrial membrane potential ($\Delta\psi_m$) (indicative for deenergization), instead of an expected elevation. In addition, in spite of a $\Delta\psi_m$ decline, a parallel stimulation of cell respiration was not seen (Thevenet et al., 2016). Remarkably, enhanced ketogenesis or lowering of the NAD(P)H to NAD(P^+) ratio or of $\Delta\psi_m$ were not observed when neurons were exposed to both medium-chain fatty acids (MCFAs). Finally, based on results obtained by respiration measurements using isolated brain mitochondria it has been hypothesized that brain mitochondria have some capacity for oxidation of fatty acids, when they are administered in a mixture together with other substrates (Panov et al., 2014). The underlying study shows that l-palmitoylcarnitine increased the phosphorylating respiration (State 3) of mitochondria oxidizing either pyruvate plus malate or glutamate plus malate by 16% or 23%, respectively. In contrast, l-palmitoylcarnitine inhibits in combination with pyruvate plus malate the uncoupled respiration, but stimulates in combination with glutamate plus malate this respiration. This indicates that secondary activities of l-palmitoylcarnitine contribute to the observed changes in the respiration.

Major support for the existence of cerebral mitochondrial β-oxidation comes from a study, where the rat brain was perfused with ^{13}C-octanoic acid (Ebert et al., 2003). β-Oxidation of octanoic acid was derived from the analysis of the ^{13}C-labeled metabolite pattern in extracts of intact brain by ^{13}C-NMR spectroscopy and was estimated to contribute up to 20% of the energy demand of the rat brain. Nevertheless, it might be questioned, whether this estimation is representative for cerebral β-oxidation of LCFAs. In contrast to the carnitine-dependent mitochondrial uptake of LCFAs, octanoic acid permeates freely across the inner mitochondrial membrane (IMM) and becomes activated to acyl-thioester by intramitochondrial ATP. Thus, it is possible that these differences between octanoic acid and LCFAs indicate a preferred degradation of octanoic acid. Our own experience sharply contrasts with the concept of a significant β-oxidation of octanoic acid in brain. First, using preparations of brain mitochondria (representing a mixture of neuronal and astrocytic mitochondria) we did not find an enhanced ROS generation after the addition of low micromolar concentrations of octanoic acid or l-octanoylcarnitine, as it can be expected from an operational β-oxidation (Schönfeld and Reiser, 2017). Even in brain mitochondria kept in a starvation situation (supplemented with only malate or malate plus ADP), octanoic acid or l-octanoylcarnitine do not induce an enhanced release of ROS. Donation of NADH and $FADH_2$ by the β-oxidation is likely to stimulate ROS generation via the respiratory chain and the combined activity of the acyl-CoA dehydrogenase plus electron transfer flavoprotein (ETF)-ubiquinone-oxidoreductase. Indeed, rat liver mitochondria (RLM) respond clearly under such conditions to octanoic acid or octanoylcarnitine with enhanced ROS generation (Schönfeld and Reiser, 2017).

Experimentally, we also examined a possible β-oxidation in rat brain mitochondria (RBM) by measuring the response of the respiration and of the membrane potential ($\Delta\psi_m$) to addition of MCFAs. For comparison, both parameters were measured in RLM. When RLM were incubated with malate (a poor hydrogen-delivering substrate) and ADP (for stimulation of phosphorylation; State 3), the respiration was low (Fig. 1A, upper pair of traces). The addition of the MCFA octanoic acid increased the respiration 4-fold and a further enhancement was seen after the addition of glutamate, which is a potent hydrogen-supplying substrate for liver mitochondria. In contrast, octanoic acid was without any effect on the respiration of RBM (lower pair of traces). A large stimulation of the respiration by pyruvate (a very potent hydrogen-supplying substrate for brain mitochondria) indicates the high hydrogen demand of RBM in this incubation. In parallel, the addition of octanoic acid to RLM enhanced the polarization state of the IMM (indicated as decline of the safranine fluorescence) in State 3 (Fig. 1B, upper trace with blue circle), whereas in RBM the polarization state did not change (Fig. 1B, lower trace). These results are in line with previous studies, which claim that brain mitochondria have a negligible or very poor capacity for mitochondrial β-oxidation (Bird et al., 1985; Yang et al., 1987).

In summary, studies done with the whole brain or neural cells indicate that the brain has some capacity of mitochondrial β-oxidation, whereas other studies with isolated brain mitochondria exclude such oxidation. To our view, this stunning discrepancy might be attributed to a different experimental strategy as well as the usage of different cerebral preparations. The developing brain has a higher enzymatic activity of enzymes of the β-oxidation than the adult one (Reichmann et al., 1988). Thus, it could be that neural cells which were prepared from the developing brain (Edmond et al., 1987) or from stem cell-derived astrocytes and neurons (Thevenet et al., 2016) exhibit mitochondrial β-oxidation. In addition, some studies estimate β-oxidation based on parameters which are only indirectly, related to the degradation of fatty acids (Ebert et al., 2003; Chen et al., 2014; Schulz et al., 2015).

2.2. Why are fatty acids spurned as fuels in the brain?

Bottlenecks responsible for the poor usage of fatty acids for the cerebral energy generation are possibly (i) a limited transport passage of fatty acids from the blood into the neural cells and/or (ii) a restricted enzymatic capacity of the degradation of fatty acids by mitochondria (see Fig. 2). With respect to the first limitation the question arises: Could a rate-limited transfer of LCFAs bound to serum proteins across the BBB into neural cells restrict the use of LCFAs as fuel? LCFAs that are mostly tightly bound to albumin, readily desorb from albumin (Hamilton, 1998) and, it has been found by applying the *in situ* perfusion techniques to rat brain that a broad diversity of LCFAs rapidly permeate through the BBB at a similar rate (Spector, 1988; Williams et al., 1997; Smith and Nagura, 2001; Ouellet et al., 2009). Thus, a slow passage of non-esterified LCFAs through the BBB is not likely to be the cause for the poor fatty acid oxidation by the brain. Instead, a limitation of the carnitine-dependent uptake of LCFAs as well as their degradation by the mitochondria is the likely cause for the minor use of LCFAs as fuel. Indeed, carnitine is essential for brain functioning (Kimura and Amemiya, 1990; Jones et al., 2010). Since data from older studies

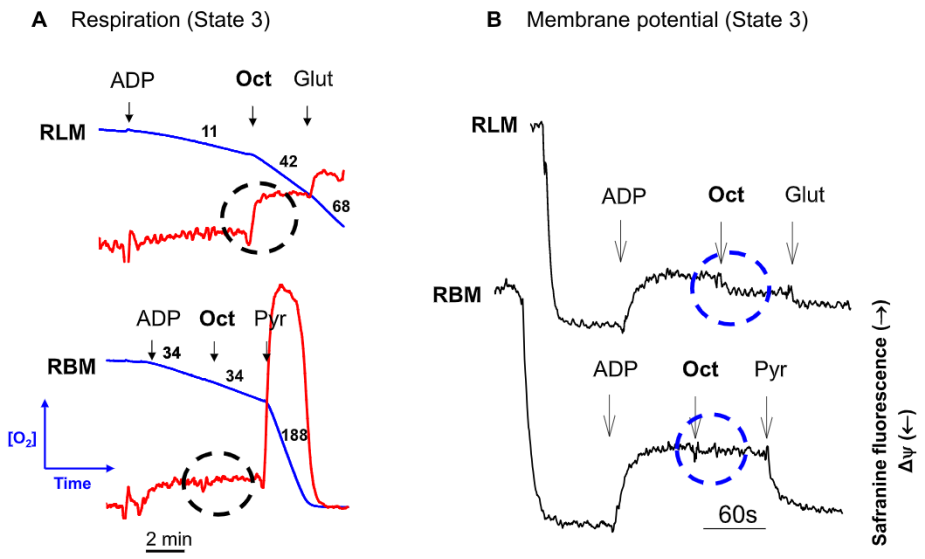

Fig. 1. Effect of octanoate on the respiration (A) and the energization (B) of brain and liver mitochondria from rat.
Mitochondria (0.5 mg protein/ml) were suspended in incubation medium (110 mM mannitol, 60 mM KCl, 60 mM Tris-HCl, 10 mM KH_2PO_4 and 0.5 mM EGTA; pH 7.4; 37 °C) supplemented with 1 mM malate. Additions, as indicated, are ADP (1 mM), octanoic acid (Oct; 0.1 mM) and glutamate (Glut; 5 mM) or pyruvate (Pyr; 5 mM). Oxygen uptake (panel A) was measured using an Oxygraph® from Oroboros Instruments (Innsbruck, Austria). Blue traces represent the decrease of the oxygen concentration in the medium, whereas red traces are the corresponding differential ratios ($d[O_2]/dt$). Rates of respiration are given as numbers (nmol O_2/min/mg of protein) at the oxygen concentration traces. Energization of mitochondria (panel B) was measured fluorimetrically using the $\Delta\psi_m$-dependent accumulation of the lipophilic cationic safranine dye (10 μM) with a Perkin Elmer Luminescence Spectrometer LS 50B (495 nm excitation, 586 nm emission). Decrease of safranine fluorescence indicates $\Delta\psi_m$-driven accumulation of the cationic safranine by energized mitochondria. Accumulation results in the aggregation of safranine molecules, thereby causing the quenching of their fluorescence. Further technical and experimental details are given elsewhere (Schönfeld and Reiser, 2006, 2017). Oct-induced changes are indicated by broken circles attached to the traces.

indicate that the activity of the carnitine palmitoyltransferase system is considerably higher in brain mitochondria than the rate of the β-oxidation of LCFAs (Bird et al., 1985), it seems unlikely that the transmembranal translocation is a limiting step for the overall degradation of LCFAs. In contrast, the recent claim that the CAT 1c, the brain-specific form of CAT 1, is not localized in mitochondria and has nothing to do with oxidation of LCFAs (Sierra et al., 2008) and exhibits no acyltransferase activity with fatty acyl-CoA derivatives and carnitine (Wolfgang et al., 2006). However, the localization of CPT 1c in the endoplasmic reticulum implicates that it has a role in a biosynthetic pathway rather than in the degradation of LCFAs. In addition, the above mentioned claim explains that the supply of brain mitochondria with the non-esterified or carnitine derivatives of octanoic or palmitic acid does not give a different mitochondrial ROS generation (Schönfeld and Reiser, 2017). When a carnitine shuttle would be involved in the degradation of these fatty acids by brain mitochondria, a higher ROS generation is expected with the carnitine derivatives. In contrast, liver mitochondria exhibit with much higher ROS generation with the carnitine derivatives.

Moreover, with l-palmitoylcarnitine as substrate, the rate of mitochondrial fatty acid oxidation by rat brain mitochondria is 50- or 30-times lower than in heart or liver mitochondria, respectively The low rate of palmitoylcarnitine oxidation has been ascribed to low specific activities of the β-oxidation enzymes present in brain mitochondria, in particular that of the last step of the β-oxidation, the 3-ketoacyl-CoA thiolase. This enzyme cleaves the C_β-C_α-bond in 3-ketoacyl-CoA intermediates by coenzyme A and, its specific activity in brain mitochondria accounts to only 0.7% of that in heart mitochondria. Consequently, low activity of the 3-ketoacyl-CoA thiolase, limits the mitochondrial fatty acid degradation in brain (see for review (Middleton, 1973)). In contrast, acetoacetyl-CoA, formed from the ketone body acetoacetate, is more rapidly cleaved into two molecules of acetyl-CoA. Acetoacetate is activated inside the brain mitochondria by the succinyl-CoA:3-oxo-acid CoA-transferase (Russell and Patel, 1982). The discrepancy between the slow cleavage of 3-ketoacyl-CoAs and an obviously rapid cleavage of acetoacetyl-CoA is explained by the existence of a succinct thiolase with much higher activity in brain (Middleton, 1973).

A severe limitation of the cerebral mitochondrial degradation of LCFAs leads to a further question: Why has evolution excluded this degradation from the cerebral tissue, as compared to liver, kidney or heart tissues? Despite being excellent fuels for the mitochondrial energy generation, supraphysiological levels of non-esterified LCFAs or their carnitine- and CoA-derivatives are the source of diverse harmful activities for cells and, especially for their mitochondria (see for review (Wojtczak and Schönfeld, 1993; Di Paola and Lorusso, 2006)). Nevertheless, despite the potential cytotoxicity of non-esterified LCFAs, tissues with a high energy-turnover comparable to that of brain (heart, kidney, liver), oxidize large amounts of LCFAs to fulfill their energy demand (Caldwell and Wittenberg, 1974).

Since the utilization of NADH and $FADH_2$ by the respiratory chain is accompanied by ROS generation, specific attention was drawn to the possibility that the fatty acid-associated ROS generation is problematic for neural cells (Speijer, 2011; Schönfeld and Reiser, 2013; Speijer et al., 2014; Speijer, 2016). Besides complexes I and III of the respiratory chain, the acyl-CoA-dehydrogenase, the ETF and the ETF-ubiquinone oxidoreductase are additional sites of the mitochondrial superoxide generation (St-Pierre et al., 2002; Lambertucci et al., 2008; Schönfeld and Wojtczak, 2008; Perevoshchikova et al., 2013). Moreover, when NADH and $FADH_2$ compete for donating their electrons to the electron acceptor ubiquinone, it is likely that the electron flux through complex I is partly impaired, thereby facilitating the leakage of electrons from complex I to molecular oxygen (Speijer et al., 2014; Speijer, 2016). Indeed, complex I is the main site of the ROS generation in the respiratory chain (Brand, 2010), and therefore, the ROS generation is most likely enhanced by that competition. Thus, it is an attractive hypothesis that a high $FADH_2$/NADH ratio is mainly responsible for

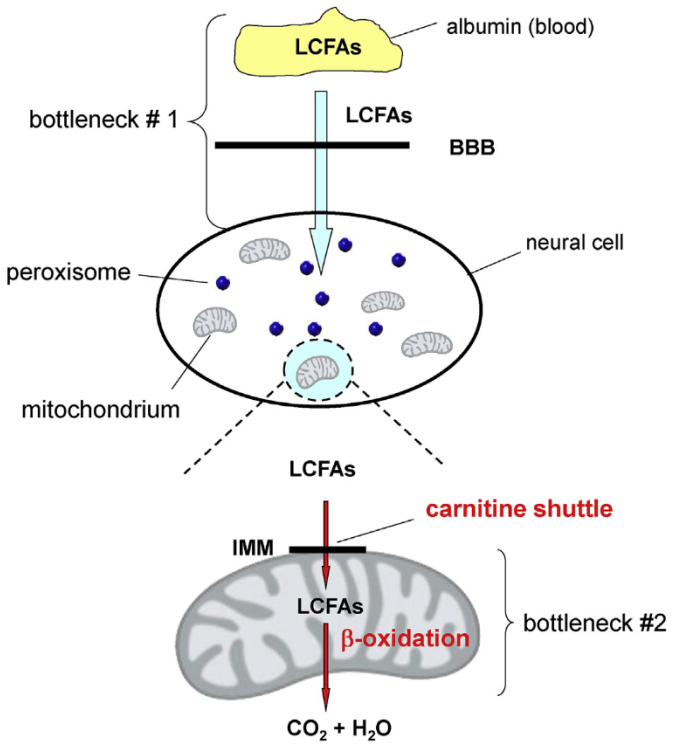

Fig. 2. Possible bottlenecks in the degradation of fatty acids by neural cells.
The transfer of serum protein-bound LCFAs into the neural cell is possibly a rate-limiting step during overall cellular degradation. This rate-limitation consists of desorption of LCFAs from serum proteins as carriers (mostly albumin) and, thereafter, the transfer of the liberated LCFAs across the BBB into the neural cell. Within the cell, LCFAs are degraded mainly by the mitochondrial β-oxidation. Peroxisomes specifically shorten VLCFAs and branched-chain LCFAs, but they also partly degrade to a small extent saturated unbranched LCFAs by peroxisomal β-oxidation. A second bottleneck is the joint processes of the transfer of LCFAs across the IMM (carnitine shuttle) and their subsequent β-oxidation. For both processes a severe limitation of the respective enzymatic capacities has been reported.

the mitochondrial ROS generation (Speijer, 2011, 2016; Speijer et al., 2014). Moreover, the $FADH_2$/NADH ratio generated by the complete oxidative degradation of palmitic acid to CO_2 and H_2O is 0.48, much higher than that generated by the complete oxidative degradation of glucose, which is 0.2. Accordingly oxidative degradation of LCFAs is the most potent mitochondrial process for ROS generation. To minimize ROS generation, obviously, brain mitochondria completely substitute LCFAs by glucose or the ketone bodies βOHB and AcAc. The latter have $FADH_2$/NADH ratios of 0.29 and 0.33, respectively.

However, why are LCFAs not spurned by mitochondria from tissues of heart, skeletal muscle or liver? To give an tentative answer to this question, it is important to recall that brain tissue has a high content of PUFAs, a relatively weak anti-oxidative defense capacity and, in addition, regionally high concentrations of redox-active transition metals catalyzing the generation of ROS (Galea et al., 2012). Taken together, these facts could explain why oxidization of LCFAs is dangerous for the functional integrity of brain tissue. In line with this view is a report substantiating that brain tissue suffers more in mild oxidative stress than liver tissue (Skalska et al., 2016). Thus, when rats were orally exposed to nanoparticles of silver for initiating mild oxidative stress, the ratio of reduced to oxidized glutathione (GSH/GSSG) decreases significantly in brain, but not in liver tissue. Lowering this ratio reflects a decrease of GSH by direct ROS scavenging and GSH conjugation with products that result from lipid peroxidation (Cnubben et al., 2001).

In summary, it is likely that the ROS generation associated with oxidative degradation of LCFAs, a high content of ROS-sensitive PUFA and a weak antioxidative defense were the reasons, why evolution equipped brain mitochondria with weak β-oxidation activity.

2.3. Medium-chain fatty acids are more than just a source of ketone bodies fuel

Despite the long-known rapid hepatic delivery of MCFAs-derived ketone bodies for the cerebral energy metabolism, recent insights indicate the use of odd-chain MCFAs as source for maintaining the level of citric acid cycle intermediates in brain mitochondria. Thus, it has been proposed that directly from heptanoic acid or from liver-supplied degradation products (β-ketopentanoic acid and β-hydroxypentanoic acid) neural cells generate propionyl-CoA, an important precursor of the formation of oxaloacetate (Willis et al., 2010; Borges and Sonnewald, 2012). Moreover, it is likely that during chronic epilepsy a seizure-associated enhanced leakage of the neurotransmitters glutamate (with α-ketoglutarate as precursor) and GABA (with oxaloacetate as precursor) takes place, thereby diminishing the levels of citric acid cycle intermediates. For that, an anaplerotic potential of odd-chain MCFAs seems to be beneficial for the treatment of drug-resistant epilepsy (Willis et al., 2010; Borges and Sonnewald, 2012; Liu and Wang, 2013; Gano et al., 2014). MCFAs (C6:0 – C12:0) differ considerably from LCFAs with respect to their oxidative degradation or harmful activities (see for a review (Schönfeld and Wojtczak, 2016). Thus, besides differences in the activation and mitochondrial uptake, MCFAs exert in mitochondria no or only weak protonophoric activity and do not significantly impair the electron transport in the respiratory chain. For the liver, and for astrocytes, MCFAs are a source of ketone bodies generation (Auestad et al., 1991). Moreover, MCFAs with either odd-chain or even-chain hydrocarbon skeletons exert different effects on the cell energy metabolism (Okere et al., 2006; Kajimoto et al., 2015). In contrast to even-chain MCFAs, β-oxidation of odd-chain MCFAs generates acetyl-CoA and, in addition, propionyl-CoA, which is an anaplerotic agent for the citric acid cycle after conversion into succinate. Indeed, it has been shown recently that triheptanoin partially restores the level of citric acid cycle metabolites in an epileptic animal model (Hadera et al., 2014). Triheptanoin is also able to attenuate harmful side-effects associated with ischemic stroke (Schwarzkopf et al., 2015). For illustration, when mice were exposed to transient ischemia, triheptanoin reduced the extracellular level of glutamate released in the mouse striatum, maintained the cellular ATP content at the desired level and prevented a decline of the respiratory activity of isolated brain mitochondria. The latter findings strongly suggest that the mitochondrial ATP regeneration is a target of the trihepatonin action (Schwarzkopf et al., 2015).

Finally, direct effects of octanoic and decanoic acid on the energy metabolism were studied in the neuronal cell line SH-SY5Y, and human neurons and astrocytes (Hughes et al., 2014; Thevenet et al., 2016). By treating the SH-SY5Y neuronal cell line with decanoic acid, a component of the medium-chain triglyceride-based ketogenic diet, it has been demonstrated that there is an increase of the activities of the citrate synthase and of complex I as well as an increase of the cellular content of mitochondria (Hughes et al., 2014). The latter has been attributed to the ability of decanoic acid to activate PPARγ (peroxisome proliterator activator receptor γ), known to stimulate mitochondrial proliferation (Kanabus et al., 2016). This explanation is supported by the lack of effect of βOHB, AcAc or octanoic acid. In conclusion, mitochondrial proliferation and, consequently, a higher capacity for the cellular energy

generation, is likely to elevate the seizure threshold in individuals with drug-resistant epilepsy (Neal et al., 2009). Additional insight has been derived from a study with neurons and astrocytes, which were treated with MCFAs (Hughes et al., 2014; Thevenet et al., 2016). From the data obtained it was concluded that MCFAs stimulate the astrocyte to neuron lactate and ketone body shuttle, and thereby improve the energy supply to neurons (see for review (Romano et al., 2017)).

3. Fatty acids with detrimental potential as causative agents of neurodegenerative diseases

Defective peroxisomal degradation of the branched-chain phytanid acid (3,7,11,15-tetramethylhexadecanoic acid), and that of the very long-chain fatty acids (VLCFAs) is associated with the pathogenesis of two prominent neurodegenerative diseases, Refsum disease and X-linked adrenoleukodystrophy (X-ALD). Phytanic acid is not formed by *de novo* synthesis of the mammalian lipid metabolism; it is mostly taken up with dairy products from ruminants (for a recent review see (Roca-Saavedra et al., 2017)).

Accumulation of VLCFAs (C22:0, C24:0, C26:0) in body fluids and tissues is the characteristic hallmark of X-ALD, but also in acyl-CoA oxidase deficiency. VLCFAs are synthesized in cells by elongation of palmitic acid by the help of a repeated four-step elongation cycle, which is catalyzed by endoplasmic reticulum membrane-embedded enzymes, and VLCFAs have unique cell-specific functions in their esterified forms (see for review (Kihara, 2012)). In healthy individuals, the degradation of both types of fatty acids is orchestrated by the peroxisomal and the mitochondrial β-oxidation pathways (see for review (van den Brink and Wanders, 2006)).

VLCFAs differ from phytanic acid by structure and hydrophobicity. Thus, phytanic acid seems to be much more mobile within the cells than VLCFAs. The latter are strongly bound to cellular proteins or incorporated into membranes. Phytanic acid operates as a protonophoric uncoupler comparable to chemical uncouplers, such as dinitrophenol, or straight-chain fatty acids (e.g., myristic or palmitic acid) (Schönfeld et al., 1997; Komen et al., 2007; Schönfeld and Wojtczak, 2007). Fatty acid-based protonophoric uncoupling is generally explained by a rapid membrane protein-assisted flip-flop movement of the protonated/deprotonated forms of long-chain fatty acids within the IMM (Skulachev, 1991). In contrast, VLCFAs are very poor protonophoric uncouplers. Moreover, when studying activities of VLCFAs on isolated mitochondria or cells, VLCFAs have to be solubilized in α-cyclodextrin-vehicles due to their very poor solubility (see (Hein et al., 2008) and references therein). Furthermore, the desorption rate of C26:0 from phospholipid membranes is about ten thousand times slower than that of long-chain fatty acids (C14:0 to C18:0) (Ho et al., 1995). Moreover, VLCFAs are markedly incorporated into different lipid species, such as in gangliosides (Garashi et al., 1976). The latter finding suggests that VLCFAs exhibit some cytotoxicity by alteration of membrane properties, including the change of ganglioside-associated immunoreactivity (Kannagi et al., 1982; Pestronk, 1991). A discussion of detrimental cellular activities of phytanic acid and that of VLCFAs has been given in comprehensive reviews (Wanders et al., 2010; De Munter et al., 2015; Fourcade et al., 2015; Schönfeld and Reiser, 2016).

Moreover, LCFAs are enriched in tissues, mostly as intermediates of ß-oxidation or its carnitine derivatives, during deficiencies of the long-chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD; (Tyni and Pihko, 1999)) or of the mitochondrial trifunctional protein (MTP; (Ushikubo et al., 1996)). LCHAD is a part of the α-subunit of the MTP, which exhibits in addition activities of 2-enoly-CoA hydratase (α-subunit) and the 3-ketoacyl-CoA thiolase (ß-subunit). Using rat cerebral homogenates and preparations of mitochondria, it has been demonstrated that long-chain 3-hydroxy fatty acids are able to induce oxidative stress and impair mitochondrial bioenergetics in rat brain (Tonin et al., 2010, 2014).

3.1. Phytanic acid impairs a diversity of energy-dependent functions

Despite being an analog of palmitic acid, the methyl group in the β-position of phytanic acid prevents the formation of a keto group, which consequently blocks the degradation of phytanic acid by the mitochondrial β-oxidation. Therefore, the degradation of phytanoyl-CoA starts in peroxisomes by converting the phytanoyl-CoA to pristanal by an α-oxidation step, and thereby shifting the methyl group to the α-position in pristanal. After conversion of pristanal to the CoA-derivative of pristanic acid (2,6,10,14-tetramethylpentadecanoic acid), three cycles of peroxisomal β-oxidation generate 4,8-dimethylnonanoyl-CoA as end-product. Now, 4,8-dimethylnonanoyl-CoA is released from peroxisomes, taken up by mitochondria, where its degradation is completed. In summary, degradation of phytanic acid yields three molecules of acetyl-CoA, three molecules of propionyl-CoA and one molecule isobutyryl-CoA.

In individuals with defective α-oxidation, non-esterified phytanic acid accumulates in tissues and blood reaching low millimolar concentrations. Here, we briefly summarize *in vitro* studies of the detrimental activities of pathologically high concentrations of non-esterified phytanic acid or pristanic acid on isolated mitochondria from brain, liver and on cultured cells. These branched-chain fatty acids potentially impair the cellular energy metabolism by the following activities: (i) Incorporation of the branched-chain hydrocarbon backbone into biological membranes disturbs the arrangement of the membrane constituents (Schönfeld and Struy, 1999). This may contribute to an increased Ca^{2+} leakage through the plasma membrane and from intracellular stores (Kahlert et al., 2005). (ii) Proton permeation across the IMM is effectively facilitated by the protonophoric activity of phytanic acid, thereby decreasing the ATP regeneration (Schönfeld et al., 2004). (iii) Binding of phytanic acid to the ADP/ATP-translocase slows down the exchange of matrix-located ATP against extramitochondrial ADP, sensitizes mitochondria for opening the mitochondrial permeability transition pore and thereby reduces the mitochondrial Ca^{2+} retention capacity (CRC) (Schönfeld et al., 2004). Further indications of an interaction of phytanic acid with functional membrane proteins are the activation of the GPR40 receptor (Kruska and Reiser, 2011) and the inhibition of the Na^{+}/K^{+}-ATPase activity (Busanello et al., 2013). (iv) An interaction of phytanic acid with distinct sites within the complexes of the respiratory chain enhances one-electron transfer to molecular oxygen, and thus stimulates ROS generation (Schönfeld and Reiser, 2006; Schönfeld and Wojtczak, 2007). ROS generation resulted in an inactivation of the aconitase (an enzyme of the citric acid cycle), a partial oxidation of the matrix glutathione pool, enhanced lipid peroxidation and protein carbonyl formation. (v) Finally, it has also been demonstrated that the activated form of phytanic acid, phytanoyl-CoA, impaired two crucial enzymes involved in the supply of reducing equivalents from glucose degradation, namely the pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase (Bunik et al., 2006). Due to the strong dependency of the brain energy metabolism on glucose, it is tempting to speculate that this inhibitory activity should be more harmful in brain as compared to other organs. It is also worth to emphasize that the detrimental activities of phytanic acid or phytanoyl-CoA observed *in vitro* and *in situ* on the cellular energy metabolism are stronger than those found with palmitic acid or its CoA-ester (Schönfeld et al., 2004; Schönfeld and Reiser, 2006; Bunik et al., 2006).

In summary, *in vitro* studies with isolated mitochondria and

various cell types (neural cells (Kahlert et al., 2005; Rönicke et al., 2009), fibroblasts (Komen et al., 2007) or smooth muscle cells (Idel et al., 2002) have uncovered that phytanic acid (including phytanoyl-CoA and pristanic acid) exert several acute harmful actions at different cellular sites. Particularly mitochondria are targets of phytanic acid. Apart from this, phytanic acid is able to activate nuclear genes, mediated by the nuclear receptors RXR- and PPARα (Lemotte et al., 1996; Ellinghaus et al., 1999). This suggests that chronic exposure to phytanic acid might contribute to the pathogenesis of Refsum diseases by alteration of the gene expression pattern.

3.2. VLCFAs exert their toxicity mostly by an alteration of membrane properties

Several studies have uncovered that in X-ALD a defective ABCD1-transporter prevents the uptake of VLCFAs by peroxisomes (Mosser et al., 1993; van Roermund et al., 2008; Wiesinger et al., 2013) and thus makes their degradation by the peroxisomal β-oxidation impossible. Subsequent to the impaired peroxisomal β-oxidation, VLCFAs are enriched in tissues and plasma, resulting in the alteration of the profile of non-esterified fatty acids and that in membrane lipids (Sandlers et al., 2012). Thus, an alteration of membrane functions has to be expected. Indeed, from *in vitro* studies it was reported that VLCFAs disrupt the membrane structure (Ho et al., 1995), enhance the micro-viscosity of the membranes of cultured human adrenocortical cells and, thereby probably reduce the ACTH-stimulated cortisol secretion (Whitcomb et al., 1988).

Moreover, muscle mitochondria isolated from Abcd1$^{-/-}$, a mouse model for X-ALD, did not show abnormalities compared to those from wild-type mice (Oezen et al., 2005). Studies with brain mitochondria from wild-type and Abcd1$^{-/-}$ mice confirm this observation (Kruska et al., 2015). Despite a substantial enrichment of VLCFAs in whole muscle homogenates of ABCD1$^{-/-}$ mice, an enrichment was not found in muscle mitochondria membranes. Nevertheless, a direct exposure of mitochondria or cells to VLCFAs clearly indicates the detrimental activity of VLCFAs. Using oligodendrocytes, neurons and astrocytes prepared from the hippocampi of wild-type rats, *in situ* measurements report a lowering of the mitochondrial $\Delta\psi_m$ by exposure to VLCFAs (Hein et al., 2008). Later reported results obtained with human neuronal cells (SK-NB-E) and astrocytes from brains of wild-type and Abcd1$^{-/-}$ confirm this finding (Zarrouk et al., 2012; Kruska et al., 2015). Furthermore, a decline of the mitochondrial $\Delta\psi_m$ combined with the VLCFA-induced increase of the intracellular Ca^{2+} level are factors that sensitize mitochondria for opening the mitochondrial permeability transition pore. This might explain that astrocytes (Hein et al., 2008) or SK-NB-E cells (Zarrouk et al., 2012) exposed to VLCFAs undergo cell death within 24 h or 48 h, respectively.

When isolated brain mitochondria were exposed to VLCFAs, an enhanced ROS generation and release of accumulated Ca^{2+} was detected (Hein et al., 2008; Kruska et al., 2015). Remarkably, brain mitochondria from wild-type control and Abcd1$^{-/-}$ mice respond similarly to VLCFAs exposure with respect to the induction of ROS generation, impaired oxidative ATP synthesis and diminished CRC (Kruska et al., 2015). Taken together, these findings suggest that the loss of the ABCD1 protein has no direct effect on mitochondrial parameters, evidenced by substrate-dependent rates of oxygen consumption, the respiratory control index and enzymatic activities within the respiratory chain (see also (Oezen et al., 2005)). However, even when the mitochondrial parameters of Abcd1$^{-/-}$ mice are not affected, a reduced content of mitochondria in Abcd1$^{-/-}$ mice was reported (Morató et al., 2013). Moreover, the response of isolated brain mitochondria depends on the hydrocarbon chain length of VLCFAs. Thus, the detrimental activity of VLCFAs on resting respiration, ATP synthesis or CRC declined from C22:0 to C26:0 (Hein et al., 2008; Kruska et al., 2015). Similar results were reported in studies using SK-NB-E cells by measuring the whole cell superoxide generation or their capacity to reduce the cell permeable tetrazolium derivative MTT to the blue colored formazan (Zarrouk et al., 2012). Inside the cells, the dye MTT is reduced mostly by electrons donated by the mitochondrial respiratory chain. Nevertheless, a potent detrimental activity of C26:0 has been claimed, as summarized recently (Fourcade et al., 2015). According to that an excess level of C26:0 initiates enhanced ROS generation, and this finding has been hypothesized to be the cause of an early-onset oxidative damage in the pathogenesis of an Abcd1$^{-/-}$ mouse model (Fourcade et al., 2008, 2014, 2015). Remarkably, oxidatively damaged proteins mostly belong to citric acid cycle, oxidative phosphorylation and glycolysis (Galino et al., 2011). In addition, human fibroblasts from healthy individuals or X-ALD patients suspended in a galactose-containing medium (to exclude glycolytic ATP generation) and incubated with C26:0, show slightly decreased phosphorylating respiration (Lopez-Erauskin et al., 2013).

Another feature of VLCFAs is their detrimental activity on the generation of reducing equivalents in the cells, as indicated by the mostly NAD(P)H-dependent reduction of the cell impermeable electron acceptor tetrazolium derivatives (WST-1) (Kruska et al., 2015). Thus, of particular interest is the finding that the reduction of the cell-impermeable WST-1 was much more impaired in VLCFAs-treated astrocytes from Abcd1$^{-/-}$ mice as compared to those taken from wild-type mice (Kruska et al., 2015). WST-1 becomes reduced by a trans-plasmamembrane transport of "NAD(P)H electrons", mainly derived from glucose degradation (Berridge and Tan, 2000). Consequently, supraphysiological levels of VLCFAs potentially diminish the glycolytic ADP regeneration. In addition, the poor reduction of extracellular WST-1 suggests that VLCFAs reduce the capacity of Abcd1$^{-/-}$ astrocytes to revert $NAD(P)^+$ (oxidized pyridine nucleotides) to NAD(P)H (reduced pyridine nucleotides).

4. Conclusions

Mitochondrial energy metabolism uses fatty acids as fuel during embryonic and early postnatal development of neural cells. Thereafter, a shift from fatty acids fueling to that of glucose takes place (Erecinska et al., 2004). According to the generally accepted view, degradation of the hydrogen-rich fatty acids by the mitochondrial β-oxidation is not or only to a negligible extent used for energy generation in the brain. Nevertheless, studies using cultured astrocytes or the whole brain indicate a moderate capacity of mitochondrial β-oxidation (Ebert et al., 2003; Chen et al., 2014; Schulz et al., 2015; Sayre et al., 2017). Further support for mitochondrial β-oxidation in brain comes from a study, where the rat brain was perfused with ^{13}C-octanoic acid (Ebert et al., 2003). In contrast, work of other researchers does not support the existence of carnitine palmitoyltransferase (CAT 1c) in mitochondria of neurons (except that in hypothalamic neurons of the arcuate nucleus) and, thus, exclude that it has a general role in the degradation of LCFAs (Wolfgang et al., 2006; Sierra et al., 2008). In addition, recent studies with astrocytes and isolated brain mitochondria clearly exclude a mitochondrial β-oxidation of octanoic acid (Schönfeld and Reiser, 2017) and this review).

Using *in vitro* and *in situ* studies, it has been repeatedly demonstrated that non-esterified LCFAs or their activated forms potentially exert a large variety of harmful side-effects on mitochondria of different tissues. In brain, these deleterious activities were uncovered after the discovery that certain enzymatic defects of the peroxisomal degradation of phytanic acid or of VLCFAs dramatically enhance the concentrations of these fatty acids. For

the latter mitochondria are sensitive targets. Nevertheless, in skeletal muscle, kidney, heart or liver, LCFAs are excellent substrates for the mitochondrial β-oxidation. Why are brain mitochondria not equipped with a similar enzymatic capacity for the degradation of LCFAs as mitochondria from noncerebral tissues? Our answer is: Neuronal mitochondria exhibit high rates of oxygen consumption, and consequently they are potent sources of ROS generation. Neuronal mitochondria are considered to be especially susceptible to oxidative stress. Moreover, brain tissue is rich in peroxidizable fatty acids and has low antioxidative defense (Galea et al., 2012). Finally, fatty acids are considered to be substrates that especially promote mitochondrial ROS generation (St-Pierre et al., 2002; Lambertucci et al., 2008).

These facts taken together suggest that the β-oxidation is highly deleterious for brain mitochondria.

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