Ketogenic diet and astrocyte/neuron metabolic interactions

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Abstract: The ketogenic diet is an anticonvulsant diet enriched in fat. It provides the body with a minimal protein requirement and a restricted carbohydrate supply, the vast majority of calories (more than 80-90%) being given by fat. Though anticonvulsant activity of ketogenic diet has been well documented by a large number of experimental and clinical studies, underlying mechanisms still remain partially unclear. Astrocyte-neuron interactions, among which metabolic shuttles, may influence synaptic activity and hence anticonvulsant protection. The astrocyte-neuron metabolic shuttles may be themselves influenced by the availability in energetic substrates such as hydrates of carbon and fats. Historically, ketogenic diet had been designed to mimic changes such as ketosis occurring upon starvation, a physiological state already known to exhibit anticonvulsant protection and sometimes referred to as "water diet". For this reason, a special attention should be paid to metabolic features shared in common by ketogenic diet and starvation and especially those features that might result in anticonvulsant protection. Compared to feeding by usual mixed diet, starvation and ketogenic diet are both characterised by increased fat, lowered glucose and aminoacid supplies to cells. The resulting impact of these changes in energetic substrates on astrocyte/neuron metabolic shuttles might have anticonvulsant and/or neuroprotective properties. This is the aim of this communication to review some important astrocyte/neuron metabolic interactions (astrocyte/neuron lactate shuttle, glutamate-induced astrocytic glycolysis activation, glutamate/glutamine cycle along with the neurovascular coupling) and the extent to which the way of their alteration by starvation and/or ketogenic diet might result in seizure and/or brain protection.

Key words: ketogenic diet, epilepsy, astrocyte/neuron shuttle, glucose, lactate, ketone bodies

Introduction

We have previously developed evaluation of anticonvulsant and neuroprotective drugs [1] along with interest in understanding molecular basis of antiepileptic activity of the ketogenic diet [2]. In a previous review, emphasis was made on relationships between the great many changes induced by ketogenic diet and some putative biological targets potentially capable of inducing anticonvulsant protection such as ATP-sensitive potassium channels and the more recently described two pore domains potassium channels [2]. In this context, the metabolic interactions existing between neurons and astrocytes were also evoked. Activation of ATP-sensitive potassium channels by ketogenic diet metabolites has been recently shown [3] although experimental support for activation of the two pore domains potassium channels is still awaited.

The present communication is aimed at focusing on the astrocyte/neuron metabolic interactions and on the way in which they might be affected by the ketogenic diet. It gives an account successively for the historical background of anticonvulsant diets, the part of ketogenic diet which provides anticonvulsant protection, the neuron/astrocyte metabolic interactions in normal feeding conditions (astrocyte/neuron lactate shuttle, glutamate-induced astrocytic glycolysis activation, glutamate/glutamine cycle), the neurovascular coupling, the neuron/astrocyte metabolic interactions upon ketogenic diet (astrocyte/neuron ketone body shuttle), the putative metabolic anticonvulsant changes induced by the ketogenic diet (increased CNS GABAergic tone and reduced glutamate availability for neurotransmission), other and concluding remarks emphasizing the convergent metabolic changes of ketogenic diet to protect brain against epilepsy.

Brief historical account for anticonvulsant diets

Evocations of dietary measures adopted to counteract seizures have been made in Biblical times and later in Middle Ages by submitting epileptic patients to "water diet" [4]. In water diet, the uptake of food is suspended and only access to water is allowed, a condition which by definition corresponds to starvation.

In the early last century, water diet was replaced by ketogenic diet in order to improve the compliance of patients to the therapeutic diet [5, 6].

The prototype nutritional profile of ketogenic diet corresponds to a diet composition of 80% fats, 15% proteins and 5% hydrates of carbon. Introduced in the 1920's, it was eclipsed later in the following years and decades due to the development of the first efficacious antiepileptic drugs, phenobarbital and phenytoin [2].

Since the last decade there has been a large regain of interest for ketogenic diet as an anticonvulsant therapy. This diet has proven efficacy in many animal seizure models and in human clinical trials [2]. Interestingly, ketogenic diet may be active against pharmaco-resistant epilepsy, and because intractable epilepsy can be an indication for brain surgery, the diet has avoided some patient to undergo chirurgical brain intervention.

Due to the emerging success of ketogenic diet in improving epileptic patients, another fat diet, the Atkins'diet, has been recently challenged as an anticonvulsant diet. This diet was introduced by Dr Atkins in the 1950's initially to counteract the development of obesity. The prototype nutritional profile of the Dr Atkins diet slightly differs in composition from ketogenic diet and is composed by 60% fats, 30% proteins and 10% hydrates of carbon. The Atkins'diet has now also proven efficacy in animal models and in human epileptic patients [7, 8]. In contrast to ketogenic diet, cooking preparations compatible with the Atkins'diet may be found in restaurants and cafeterias, and this is a factor which may help patient to comply the diet. Modified Atkins'diets are currently evaluated in terms of threshold for glucose supply allowed to maintain antiepileptic protection [8].

Another dietary measure which presents with preliminary experimental anticonvulsant prop-

erties is caloric restriction [9]. Finally, some other diets that have been proposed to exhibit anticonvulsant potentialities, as for instance gluten-free diet [10, 11] remain to be investigated on a large scale.

What is part of ketogenic diet giving anticonvulsant protection?

In contrast to several protective and preconditioning effects induced by fats in which protection may be given by individual fatty acids (alpha-linolenic acid and tolerance to stroke and epilepsy) [12] there has been until now a failure to demonstrate that the anticonvulsant protection results from the presence of individual fatty acids in the diet. Instead, anticonvulsant protection might be linked to the metabolic state induced by ketogenic diet. Though metabolic changes induced by ketogenic diet on astrocyte/neuron metabolic interactions account for only part of the pleiotropic effects of ketogenic diet, these changes, in which neuronal glucose or lactate oxidation are shifted towards ketone body utilization, include convergent anticonvulsant mechanisms as developed below in the following sections. Nevertheless, supplementation with specific fatty acids such as ω-3 fatty acids has been recommended to protect the cardiovascular system against the hyperlipaemia associated to ketogenic diet [13].

Neuron/astrocyte metabolic interactions in normal feeding

In normal feeding conditions, glucose represents the main energetic fuel for brain metabolism. Moreover, brain glucose consumption is about 20-25% of total body glucose consumption. The glucose may be oxidized by both neurons and astrocytes. The possibility also exists for part of glucose being partially oxidized in astrocytes to complete its oxidation in neurons, using lactate as a shuttle between astrocytes and neurons (astrocyte/neuron lactate shuttle), glutamate being capable of boosting astrocytic glycolytic activation in the same time as taking place in the neuron/astrocyte glutamate/glutamine cycle.

For neuronal/astrocyte metabolic interactions presented in this section and the following section Neurovascular coupling), the reader may be referred to valuable information given elsewhere on metabolite shuttles and neurovascular coupling [14-17] or on glutamate/glutamine cycle [18, 19].

The astrocyte/neuron lactate shuttle

Figure 1 represents a classic neuro-glial-vascular unit and stresses the metabolic supply and utilization of glucose. Glucose may be transferred from the blood stream to the astrocytes thanks to the successive interventions of endothelial and astrocytic plasma membrane GLUT 1 (Glucose Transporter 1). In the astrocyte, glucose is submitted to glycolysis with as a net result the formation of pyruvate, NADH and ATP. To proceed to completion, glycolytic glucose oxidation needs recovery of NAD$^+$ from NADH. This NADH oxidation is performed during the oxidation of pyruvate to lactate catalyzed by LDH5 (Lactico-DeHydrogenase 5). The resulting lactate may be then transferred from the astrocyte to the neuron, and this occurs upon intervention of astrocytic and neuronal plasma membrane MCT (MonoCarboxylate Transporters) 1 and 2, respectively. On the other hand glucose can enter the neuron *via* neuronal plasma membrane GLUT3. Both glucose and lactate are energetic fuel for neuronal metabolism. Lactate is converted back by

LDH1 to pyruvate which may be also formed by glycolysis from glucose. Lactate- and glucose- derived pyruvate can enter mitochondria to be oxidized by pyruvate dehydrogenase to acetyl-CoA units further oxidized in the Krebs'cycle. Glycolytic (cytosolic) and total (cytosolic plus mitochondrial) oxidations of glucose lead to the formation of 2 and 36-38 ATP, respectively.

When the neuron is supplied in both lactate and glucose, it oxidizes preferentially lactate. This preference results from the competition existing between lactate dehydrogenase and glycolytic glyceraldehyde-3-phosphate for cytosolic NAD$^+$. This competition is in favour of lactate dehydrogenase, explaining why, when available, lactate is preferentially used by the neuron.

The glutamate-induced astrocytic glycolysis activation

As mentionned above, astrocytic glycolysis needs NAD$^+$ recycling from NADH, an oxidative reaction ensured by astrocytic lactate
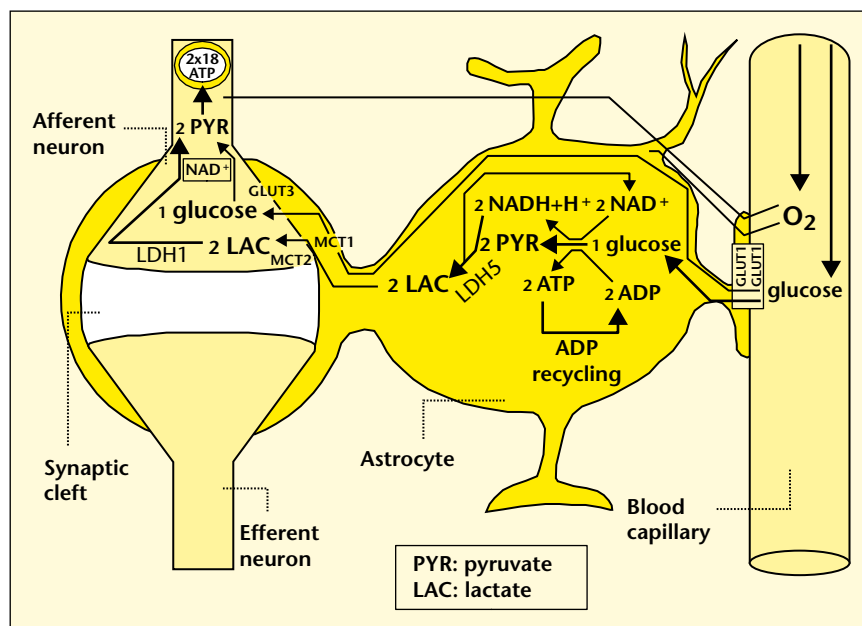

Figure 1. *Astrocytic/neuronal glucose oxidation and lactate shuttle. The biological interactive compartments include the blood vessel (right panel) , the synapse with the afferent and efferent neurons (left panels), and the astrocyte (central panel) with a typical end feet in connexion with the blood vessel and an extension which envelops the synapse and contributes to the tripartite synapse, a current trend in neurobiology. Blood glucose may enter the astrocyte via endothelial and astrocytic glucose transporters 1 (GLUT1), the entry in the neuron further requiring neuronal glucose transporter 3 (GLUT3). Within the astrocyte, part of glucose may be stored as glycogen via glycogenesis (event not shown on the figure) whereas conversion to pyruvate occurs via cytosolic glycolysis. This glucose oxidation results in NADH and ATP productions. To procede to completion, astrocyte glycolysis needs re-cycling of cytosolic NAD$^+$ and ADP. Recovery of astrocyte cytosolic NAD+ is ensured by LDH5 (Lactate DeHydrogenase 5) which converts pyruvate to lactate further exported in the neuron via astrocytic MCT1 (MonoCarboxylate Transporter 1) and neuronal MCT2 in which metabolism is directed towards mitochondrial pyruvate oxidation. Recovery of ADP can occur during glutamatergic neurotransmission according to events depicted in figure 2.*
Astrocyte to neuron shuttle of lactate occurs for both pre-synaptic (illustrated) and post-synaptic (not shown) neurons. This type of shuttle classically involves an extracellular pool of metabolites (astrocytes → extracellular space → neuronal shuttle). Other comments are in the text.

dehydrogenase which converts pyruvate and NADH into lactate and NAD$^+$. To proceed to completion, astrocytic glycolysis also needs ADP recycled from ATP. Such a recycling reaction occurs upon synaptic glutamate neurotransmission *(figure 2)*. After release by the presynaptic neuron of glutamate in the synaptic cleft, this neurotransmettor stimulates postsynaptic (and astrocytic) ionotropic (NMDA and AMPA receptors) and metabotropic receptors. Subsequent removal of excess glutamate from synaptic cleft involves presynaptic and astrocytic uptake mechanisms. The astrocytic glutamate uptake is by far more active than the presynaptic re-uptake.

Glutamate uptake by the astrocyte is associated to the concomitant entry of sodium ions (Na$^+$). This entry in cells of both sodium ions and glutamate catalysed by the astrocytic glutamate transporters (for a review on astrocytic glutamate transporters, the reader may be referred to the review of Anderson and Swanson [20]) triggers a boost in cytosolic ATP hydrolysis to ADP and therefore induces a huge increase in ADP availability for glycolysis. The mechanisms are as follows and detailled in *figure 2*. The amount of sodium having entered the astrocyte is extruded outside the cell through the action of the Na$^+$/K$^+$ATPase which catalyzes the entry of 2 K$^+$ against the exit of 3 Na$^+$ along with hydrolysis of 1 ATP and which then represents a first site for regenerating ADP from ATP. Astrocytic glutamate (plus ammonium) is converted to glutamine by glutamine synthase in a reaction which consumes one molecule of ATP and which represents a second site for regenerating ADP from ATP. So, astrocytic handling and metabolism of glutamate may increase hugely the cytosolic ADP/ATP ratio and hence the glycolytic rates (glucose to pyruvate conversion) and subsequent formation of lactate, addressing of which to the neurons provides an immediate important source of metabolic fuel. This fuel supply is devoted to help neurons to face the energetic demand required for restoration of ionic gradients previously modified by the action potential propagation, for synthesis of new neurotransmitter and their vesicles and for the forecoming neurotransmitter release which all represent energy expensive processes. A last remark in this subsection concerns the stoechiometry between astrocytic glutamate uptake and astrocytic glucose oxidation. Glucose entering the glycolysis pathway was thought for a long time to be linked to glutamate uptake in a 1/1 stoechiometric ratio because as mentioned above astrocytic glutamate uptake generates 2 ADP from 2 ATP, and glycolyis 2 ATP from 2ADP. However, recent evidence indicates that glutamate may be itself a energetic substrate for the astrocyte, therefore escaping ATP-consuming glutamine conversion and generating on the opposite ATP production, thus lowering the astrocytic glucose oxidation/glutamate uptake ratio.

The glutamate/glutamine shuttle

The astrocytic ATP-consuming glutamate synthase mentioned above allows the conversion of glutamate to the neuro-inactive compound glutamine. Glutamine may be transferred back to neurons as a glutamate precursor. Indeed, in neurons it may be converted back to glutamate by a phosphate-dependent glutaminase. The resulting newly formed glutamate can then be stored in vesicles for future release in the synaptic cleft. As mentioned above, its release in the synaptic cleft induces post-synaptic (and astrocytic) receptor activation, and its subsequent uptake by astrocytes generates a pool of intracellular glutamate available for glutamine conversion. This astrocytic glutamate to glutamine conversion followed by glutamine export to the neuron in which glutamate is regenerated back from glutamine, is "vesicled" and released in the synapse, and subsequently up-taken by the astrocyte represents a metabolic cycle referred to as the "glutamate/glutamine cycle".

The neurovascular coupling

In the same time as synaptically released glutamate which is up-taken by the astrocytes boosts metabolism in these cells, it also stimulates metabolic exchanges and supply involving the blood capillary *via* the induction of a local vasodilation. This phenomenon is referred to as neurovascular coupling, it is depicted in *figure 3* and has been reviewed and commented by Bonvento, Sibson and Pellerin [14].

Neuron/astrocyte metabolic interactions in high fat diet

As mentioned above, in high fat diet, fatty acids represent the vast majority of energetic substrates and, simply stated, glucose supply is not far from being turned off. In these conditions, fatty acids represent the main energetic fuel for the whole body. In this respect, the main energetic fuel oxidized by neurons is derived from fatty acids and is represented by ketone bodies (β-hydroxybutyrate, acetoacetate, acetone). The precise mechanisms by which these ketones are exactly supplied to neurons have
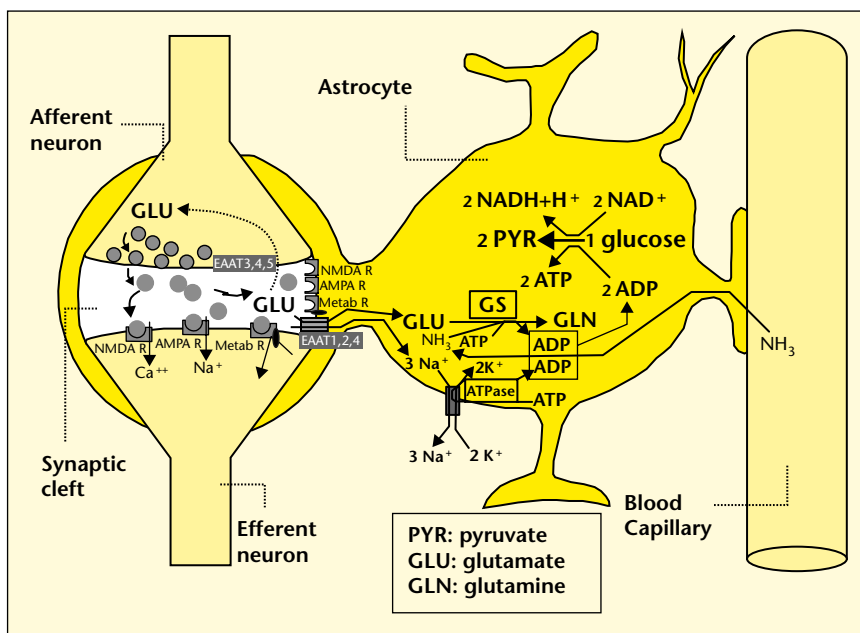

Figure 2. *Glutamate-induced astrocyte cytosolic ADP formation and subsequent glycolysis activation - Mechanisms leading to an increased ADP/ATP ratio in the astrocyte cytosol, a metabolic event which stimulates local glycolysis, involve the release of glutamate in the synaptic cleft with consequent activation of post-synaptic and astrocytic membrane ionotropic (NMDA and AMPA receptors) and metabotropic receptors and subsequent removal of glutamate mainly by astrocytic glutamate uptake catalyzed by EAAT 1, 2 and/or 4 (Excito Amino Acid Transporters 1, 2 and/or 4) and to a little extent by pre-synaptic EAAT 3, 4 and/or 5. One glutamate is up-taken by astrocyte EAATs in the same time as 3 Na$^+$. Further astrocytic disposition of both the metabolite and the electrolyte generates ADP from ATP through reactions catalyzed by glutamine synthase (GS) and Na/K.ATPase (ATPase), respectively. This gives rise to a huge increase in astrocyte cytosolic ADP/ATP ratio which results in a boosting of astrocyte glycolysis. Other comments are in the text.*
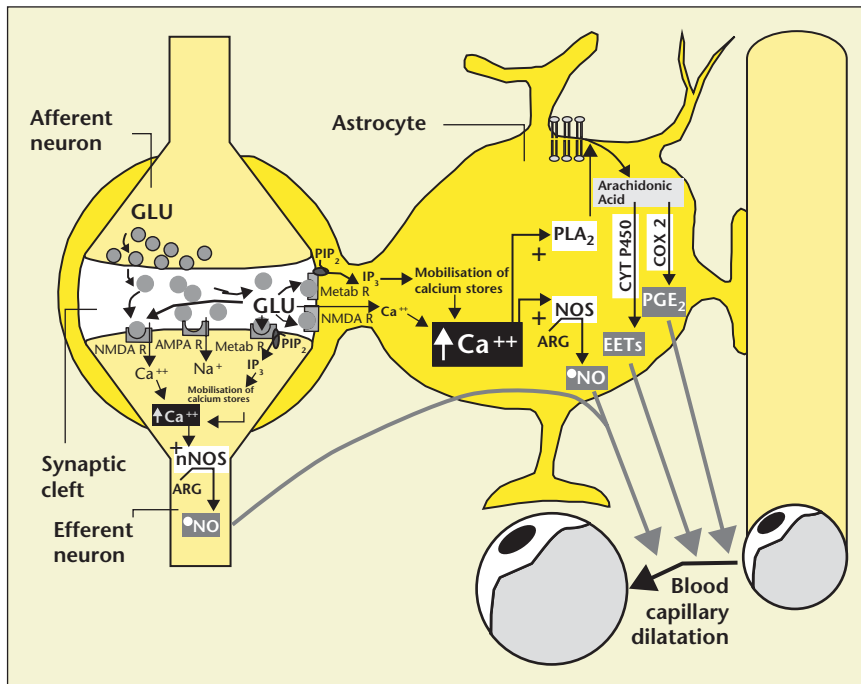
Figure 3. *Glutamate-mediated neurovascular coupling. As illustrated on Figure 2, glutamate via induction of a high astrocytic cytosolic ADP/ATP ratio can boost astrocyte glycolysis. This leads to a stimulation of events depicted in figure 1, namely increased astrocyte pyruvate and lactate formation rates and hence increased lactate supply to neurons with enhanced neuronal metabolism. The metabolic/energetic astrocyte activation needs concomitantly some adaptation in the local blood/astrocyte exchanges in order to provide substrates and to remove metabolic end-products in an adequate way. This figure 3 illustrates mechanisms allowing glutamate released in the synaptic cleft to induce this local vasodilatation through convergent routes involving activation of post-synaptic and astrocytic glutamate receptors and subsequent rise in cytoplasmic Ca++ concentration.*

Glutamate activation of post-synaptic receptors leads to increased cellular uptake (NMDA receptor) and increased intracellular mobilization (stimulation of metabotropic receptors causing release of inositol-triphosphate [IP3] from the phospho-inositol bisphosphate [PIP2] moiety of membrane phospholipids mobilizes cellular calcium stores) of Ca++. Comparable mechanisms also mediate a glutamate-induced rise in astrocytic cytoplasmic Ca++ levels. In the postsynaptic neuron, the enhanced Ca++ levels activate neuronal oxide synthase (nNOS) and hence increase ●NO formation from arginine. The nitric oxide synthase (NOS) of the astrocyte is also activated by cytoplamsic rise in Ca++. The enhanced ●NO formation originating from both the postsynaptic neuronal and astrocytic metabolism is a first mechanism for blood vessel relaxation (note that ●NO is a highly diffusible gas compound and its relaxing properties on the vasculature results from its interaction with guanylate cyclase). A second mechanism holds in the activation of astrocytic phospholipase A2 (PLA2) which stimulates release from membrane phospholipids of arachidonic acid. Upon action of cytochrome P450 (cyt P450) and cyclooxygenase 2 (COX2), arachidonate gives rise to EETs (epoxyeicosatrienoic acids) and prostaglandin E2 (PGE2), vasoactive metabolites inducing additional blood vessel relaxation.

been recently revisited [21]. Until recently, neuronal supply in ketones was considered to result from blood delivery of ketone bodies produced by liver. Hepatic production of ketone bodies from fatty acids involves two main successive pathways: fatty acid oxidation to acetyl-CoA units (mitochondrial β-oxidation) and synthesis of ketone bodies from these acetyl-CoA units (mitochondrial ketogenesis). This classic view was based on the prerogative of liver (plus kidney and intestine) to catalyse ketogenesis. Nevertheless, it was recently realised that astrocytes, like hepatocytes, also contain the mitochondrial enzymic equipment required for catalyzing not only fatty acid oxidation but also ketogenesis [21].

This has led to propose the existence of an active astrocyte/neuron ketone body shuttle model [21] in which fatty acids are supplied from blood to astrocytes to be intramitochondrially oxidized in these cells in order to generate energy covering their needs and in order to generate ketone bodies, energetic fuel preferentially utilized by neurons although to a lesser extend by astrocytes.

Putative anticonvulsant changes induced by ketogenic diet

Extensive consideration of many putative anticonvulsant mechanisms were reviewed in detail elsewhere [2, 22]. Focusing on the metabolic interactions between astrocytes and neurons, two mechanisms were proposed by Yudkoff and coworkers: increased cerebral GABAergic tone and decreased glutamatergic tone [23, 24].

The increased GABAergic tone [24] is explained by the fact that in ketogenic diet-induced neuronal oxidation of ketone bodies (3-hydroxybutyrate and subsequently acetoacetate) the entirety of these metabolic substrates is readily converted to acetyl-CoA. In contrast, in glucose feeding-induced neuronal oxidation of glucose and lactate, a portion, even if limited, of the metabolic substrates do not readily recover as acetyl-CoA, being "buffered" in the form of lactate or as glycolytic intermediates. The higher rates of acetyl-CoA formation from ketone bodies *versus* lactate plus glucose have been proposed to "aspirate" in the Krebs'cycle oxaloacetate for condensation with acetyl-CoA and further metabolism. As a consequence, lesser oxaloacetate becomes available for the reversible conversion of oxaloacetate and glutamate into α-ketoglutarate and aspartate catalyzed by aspartate/glutamate transaminase. A support to this view has been given by showing that acetoacetate lowered aspartate formation rate in cultured astrocytes incubated with glutamate [24]. Importantly, it was shown also on synaptosomes that more glutamate was consequently available for GABA synthesis, acetoacetate being finally shown to increase GABA levels [24]. The putative dynamics of these events are illustrated on *figure 4*.

In the same time as a higher amount of glutamate is directed towards GABA formation (see the preceding paragraph), it has been proposed that less glutamate was available for glutamatergic neurotransmission [23, 24]. Two mechanisms involving the escape of metabolites from the glutamate/glutamine cycle for notably removal in blood have been proposed in ketotic states *(figure 5)*. The increase of blood leucine (starvation) has been proposed to enhance the leucine/glutamine exchange between blood capillary and astrocyte with as a result increased astrocytic leucine levels and blood glutamine removal [24]. The other mechanism results from an increase in the ratio of brain on blood levels in alanine but not in other aminoacids such as glutamine and leucine (starvation and ketogenic diet *versus* normal feeding) [23]. In these conditions, it has been proposed that the abnormally high gradient of alanine concentrations existing between brain and blood should favour brain to blood transit of this aminoacid in starvation and high fat diet [23]. This tendency for a removal of alanine out of the astrocyte (to blood) has been suggested to displace the
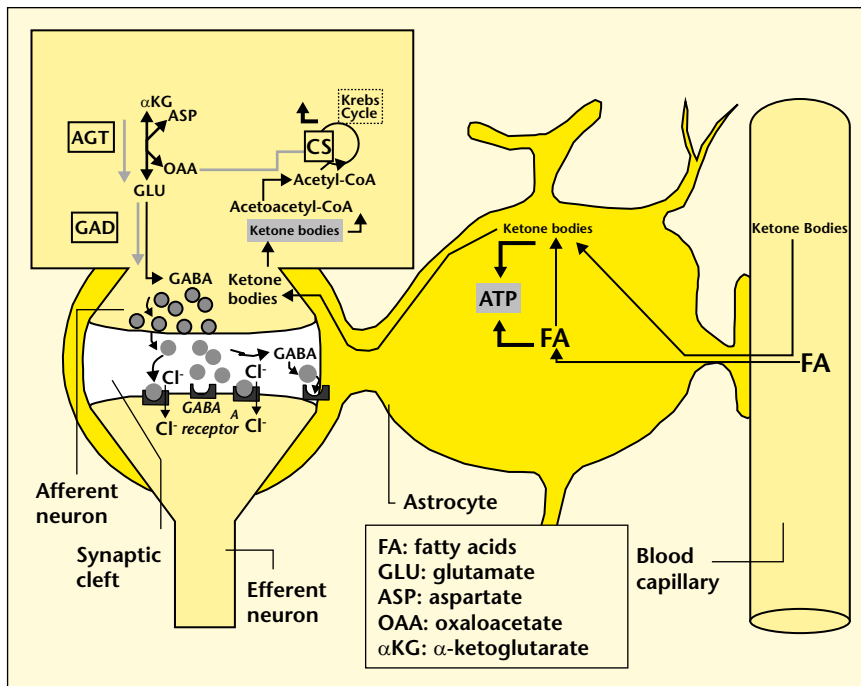
Figure 4. *Ketogenic diet – induced mechanisms that increase GABA synthesis and neurotransmission in brain. Ketone bodies supplied to neurons are accounted for by two metabolic routes, blood ketones of hepatic origin and astrocytic ketone body formation from blood fatty acids, without considering what route is here prominent. Neuronal mitochondrial oxidation of ketones to acetyl-CoA has been proposed to mobilize oxaloacetate in the Kreb's cycle at the step catalysed by citrate synthase (CS). This induces a shift in the equilibrium of the reaction catalysed by aspartate-glutamate transaminase (AGT) in favour of glutamate formation. Subsequent stimulation of glutamate decarboxylation to GABA is catalysed by GAD (Glutamic Acid Decarboxylase) and leads to enhanced GABA synthesis and neurotransmission as a brain anticonvulsant mechanism.*
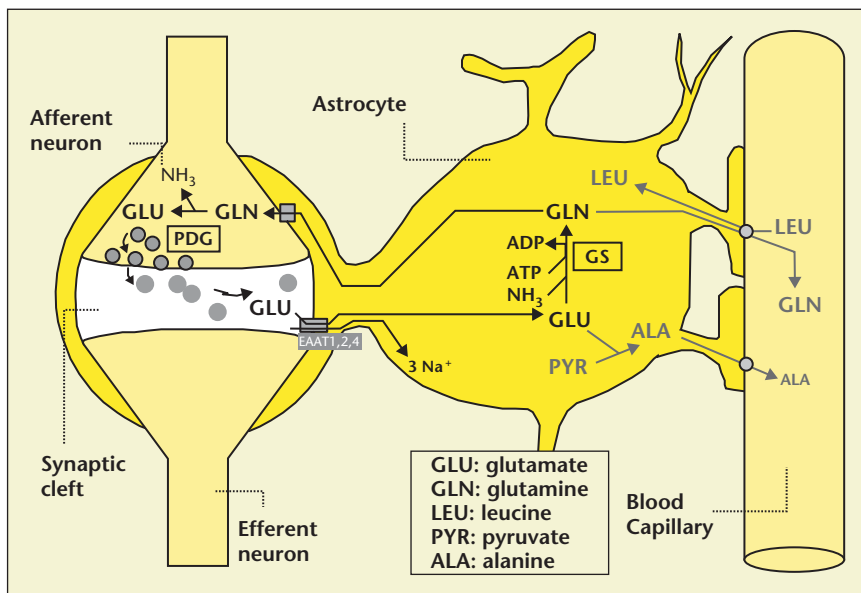

Figure 5. *Ketogenic diet (and starvation) – induced mechanisms that lower glutamate neurotransmission in brain. Although upon ketogenic diet (and starvation) a larger portion of glutamate is converted to GABA in the brain (figure 4), a part of glutamate and/or glutamine escapes the glutamate/glutamine cycle and may be lost or removed from the astrocyte to blood. In these diet conditions, more astrocytic glutamine would be removed in blood by exchange with leucine whose blood levels are increased in starvation [24] and more glutamate would also be lost as a result of increased brain to blood ratio affecting selectively alanine having a push effect on removal of this aminoacid in blood and hence on pyruvate transmination to alanine, a conversion proposed to be associated to glutamate consumption [23].*

equilibrium of glutamate/alanine transaminase in favour of glutamate (plus pyruvate) conversion into alanine (plus α-ketoglutarate), inducing a leak of glutamate from the glutamate/glutamine cycle and hence some loss of glutamate for glutamatergic neurotransmission [23].

Concluding remarks and perspective

Though many properties of ketogenic diet have been proposed to account for anti-epileptic activity, astrocyte/neuron metabolic interactions (astrocyte/neuron lactate shuttle, glutamate-induced astrocytic glycolysis activation, glutamate/glutamine cycle, astrocyte/neuron ketone body shuttle) also appear to be affected in dictinct degrees by ketogenic diet and in some way to contribute to anti-convulsant protection. In normal feeding, astrocyte/neuron metabolic interactions result in supplying neurons with lactate and/or glucose as a metabolic fuel. In high fat diet, ketone bodies represent the main metabolic fuel for neurons. Although ketone bodies are available from the blood stream in ketogenic diet, local astrocytic genesis of ketone bodies from fatty acids of blood origin which also are available in large amounts might be the preponderant mechanism for neuronal supply in ketones. Neuronal ketone metabolism has been proposed to generate acetyl-CoA in a way mobilizing oxaloacetate more intensively than when acetyl-CoA is formed during the course of lactate and/or glucose metabolism. This enhanced mobilization of oxaloacetate in the Krebs'cycle influences neuronal glutamate /aspartate transamination reaction in favour of glutamate formation, and hence increases neuronal GABA production (by decarboxylation of glutamate). On the other hand, if more glutamate provides more neuronal GABA in brain upon ketogenic diet, less glutamate is however available for glutaminergic neurotransmission as a result of a astrocytic leak from the glutamate/glutamine cycle of either glutamine or glutamate which are more abundantly than normally removed in blood or consumed consequently to an increase in blood leucine or in brain to blood ratio in alanine. Along with the antioxidant activity of ketones [25] and modifications of NADH-related signaling pathways recently described for glucose deprivation associated to ketogenic diet [26] all the presently reviewed impacts of the diet on the neuron/astrocyte metabolic interactions may be concluded to be convergent at the point-of-view of anti-seizure activity, making by otherwise the mimic of modifying these interactions a valuable goal for pharmaceutical intervention.

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