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Review

Tissue specificity of mitochondrial glutamate pathways and the control of metabolic homeostasis

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ABSTRACT

Glutamate is implicated in numerous metabolic and signalling functions that vary according to specific tissues. Glutamate metabolism is tightly controlled by activities of mitochondrial enzymes and transmembrane carriers, in particular glutamate dehydrogenase and mitochondrial glutamate carriers that have been identified in recent years. It is remarkable that, although glutamate-specific enzymes and transporters share similar properties in most tissues, their regulation varies greatly according to particular organs in order to achieve tissue specific functions. This is illustrated in this review when comparing glutamate handling in liver, brain, and pancreatic β-cells. We describe the main cellular glutamate pathways and their specific functions in different tissues, ultimately contributing to the control of metabolic homeostasis at the organism level.

1. Introduction

Glutamate is a multifunctional amino acid. Apart of being a building block for proteins [1], glutamate is also a major neurotransmitter in neuronal and non-neuronal cells [2,3], a precursor of the tricarboxylic acid (TCA) cycle and metabolic intermediate [1], an intracellular messenger [4], a key member of ammonia metabolism [5], and the inducer of one of the five basic tastes, *i.e.* the savoury taste umami [6]. This key amino acid is closely associated with mitochondrial metabolism. Mitochondrial enzymes glutamate dehydrogenase (GDH), aspartate aminotransferase, and glutaminase participate to breakdown and generation of glutamate [7].

Because various functions are associated with glutamate, it is intriguing how different tissues can achieve specific control of glutamate pathways to meet their specific tasks. In this review, we describe the main players involved in cellular glutamate metabolism. In parallel, specific glutamate-dependent functions are illustrated by comparing three important tissues playing a key role in the control of metabolic homeostasis, i.e. pancreatic β-cells, liver, and brain.

Mitochondria, and the TCA cycle in particular, represent the major metabolic crossroad enabling fuel oxidation as well as provision of building blocks, or cataplerosis, for lipids and proteins [1]. Glycolytic product pyruvate enters mitochondria and is preferentially oxidised to acetyl-CoA by pyruvate dehydrogenase. Pyruvate dehydrogenase is an important site of regulation as, among other effectors, the enzyme is activated by elevation of mitochondrial Ca^{2+}[8] and, conversely, its activity is reduced upon exposures to excess fatty acids [9]. Oxaloacetate, produced by malate dehydrogenase or alternatively by the anaplerotic enzyme pyruvate carboxylase, condenses with acetyl-CoA forming citrate, which undergoes stepwise oxidation and decarboxylation yielding α-ketoglutarate (2-oxoglutarate). The TCA cycle is completed *via* succinate, fumarate, and malate, in turn producing oxaloacetate (Fig. 1). The fate of α-ketoglutarate is influenced by the redox state of mitochondria. Low NADH to NAD^+ ratio would favour further oxidative decarboxylation to succinyl-CoA as NAD^+ is required as co-factor for this pathway. Conversely, high NADH to NAD^+ ratio would promote NADH-dependent reductive transamination through glutamate dehydrogenase (GDH), thereby forming glutamate as a cataplerotic product of the TCA cycle [1]. Alternatively, glutamate can originate from glutamine via glutaminase activity and also through transamination reactions (Fig. 1).

Glutamate can enter various metabolic pathways according to cellular demand and tissue specific function requirements. Glutamate is necessary for protein synthesis and it forms the TCA cycle intermediate α-ketoglutarate. In skeletal muscles, glutamate is used as an anaplerotic precursor for the TCA cycle to enhance oxidative metabolism [10]. Glutamate can also be taken up by secretory vesicles in excitable tissues; it initiates urea cycle via ornithine formation; and it can form γ-glutamyl-cysteine, the precursor of glutathione (γ-glutamyl-cysteine-glycine) implicated in redox control. Glutathione synthase deficiency is associated with central nervous system

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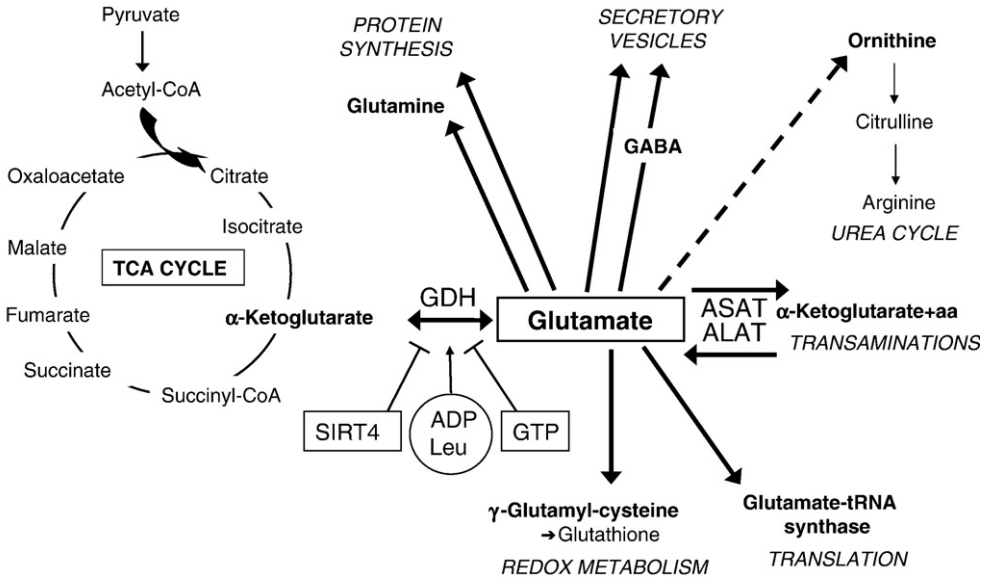

Fig. 1. Simplified scheme depicting major glutamate pathways. Glutamate dehydrogenase (GDH) is activated by ADP and L-leucine (Leu) and inhibited by GTP and SIRT4. Alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) also participate to glutamate breakdown and synthesis. Ornithine is formed from glutamate through three reactions (hashed line). See text for description.

damage and recurrent bacterial infections [11], stressing again the implication of glutamate pathways in numerous functions and tissues.

2. Glutamate dehydrogenase (GDH)

Glutamate dehydrogenase (GDH) is widely distributed throughout the eukaryotic, eubacterial and archaebacterial kingdoms with only few organisms being known to lack this class of enzymes. GDH is of major significance because it occupies a pivotal position between carbon and nitrogen metabolism. There are three basic types of GDH: those that are cofactor-specific for NAD (EC 1.4.1.2), those that are specific for NADP (EC 1.4.1.4), and dual coenzyme-specific GDHs that can use either cofactors (EC 1.4.1.3), the latter one being the most relevant for mammals.

GDHs are homopolymers consisting of two to six subunits of molecular weight of 40 to 60 kDa, the most common number of subunits being six. The GDH from bovine liver is undoubtedly the most extensively studied one [12]. In mammals, this enzyme is a homohexamer located in the mitochondrial matrix. GDH catalyses the reversible reaction α-ketoglutarate + NH_3 + NAD(P)H ↔ glutamate + $NAD(P)^+$ [12]. High GDH enzymatic activity has been found in several mammalian tissues including liver, brain, kidney, heart, pancreas, ovaries, and lymph nodes [13]. In the brain, GDH plays a key role in the cycling of the neurotransmitter glutamate between neurons and astrocytes [14]. GDH is also of major importance for ammonia metabolism and detoxification, mainly in the liver and kidney [15]. In pancreatic β-cells, the importance of GDH as a key enzyme in the regulation of insulin secretion has been recognized long ago [16]. Inhibition of GDH activity was shown to decrease insulin release [17–19], while activating mutations have been associated with a hyperinsulinism syndrome [20,21].

2.1. Enzymatic regulation of GDH

The enzyme is allosterically regulated by leucine, pyridine, adenine and guanine nucleotides [22,23]. Each subunit of GDH exhibits specific binding domains: the catalytic domain, the adenine and guanine nucleotide regulatory domains, the reduced coenzyme regulatory domain, and the ADP-ribosylation domain [12,24–27]. In most vertebrates GDH is inhibited by GTP and activated by ADP, but there are differences in the way the enzyme behaves towards the purine nucleoside phosphates [12]. GTP reduces enzyme turnover by increasing the affinity of GDH for the reaction product [28]. In contrast, ADP activates GDH by facilitating product release [28,29]. From the proposed locations of GTP and ADP binding sites in bovine GDH structure, it was suggested that these allosteric regulators exert their effects by changing the energy required to open and close the catalytic cleft during the enzymatic turnover [23,30].

Mutagenesis and photoaffinity labelling identified Lys-450 residue as a GTP binding site on GDH [31]. The region of 20 amino acids, corresponding to residues Ile-444 to Arg-463, has been suggested to encode the putative GTP allosteric domain of the enzyme [23]. The NADH binding domain of the enzyme was identified in residues Cys-270 through Lys-289 [32], residue Glu-279 playing an important role for efficient binding of NADH [33]. Analyses of the X-ray structure of bovine liver GDH indicate that Glu-275, corresponding to Glu-279 in human enzyme, forms a hydrogen bond with the coenzyme NADH [23,30]. The adenine binding domain of the ADP site within the human enzyme was identified by cassette mutagenesis and photoaffinity labelling at the Tyr-187 position [33].

Finally, GDH is also regulated by reversible cystein-specific ADP-ribosylation in mitochondria. Inactivation of the enzyme is caused by ADP-ribose association, which is suppressed by NAD(P)H. The stoichiometry between incorporated ADP-ribose and GDH subunits suggests that modification of one subunit per catalytically active homohexamer causes the inactivation of the enzyme [27]. Residue Cys-119 might have an important role in the regulation of hGDH isoenzymes by ADP-ribosylation [34]. SIRT4, a mammalian sirtuin with mitochondrial ADP-ribosyltransferase activity, is a matrix protein and becomes cleaved at amino acid 28 after import into mitochondria [35]. Recently, physiological importance of GDH regulation by ADP-ribosylation has been demonstrated. Indeed, mitochondrial ADP-ribosyltransferase SIRT4 downregulates GDH activity in pancreatic β-cells and thereby modulates insulin secretion [35,36].

2.2. Genetics of GDH

GDH is encoded by a well-conserved 45 kb gene named *GLUD1*, which is organised into 13 exons [37]. While *GLUD1*is expressed in several tissues [13], its isoform *GLUD2* is restricted to hominoids and specific for nerve tissues such as brain and retina [38]. *GLUD2* is an X-linked intronless gene [38] and was originated by retrotransposition

of a spliced mRNA derived from the intron-containing *GLUD1* gene in the hominoid ancestor less than 23 million years ago. The amino acid changes responsible for the unique brain-specific properties of the enzyme derived from *GLUD2* occurred during a period coinciding with an increase in brain size in both human and great ape ancestor [39]. *GLUD2* might have participated to enhanced brain function in humans and apes by allowing higher neurotransmitter flux and clearance. Of interest, the GDH gene was one of two candidates identified in a systematic screen for genes upregulated during late memory formation in rats [40]. Thus, *GLUD2* might have been important during evolution for increased cognitive capacities in hominoids [41].

Significance of GTP mediated regulation of GDH is highlighted through human genetic and pathophysiology. Identification of an unusual hyperinsulinism/hyperammonaemia syndrome has been associated with dominant mutations in GDH that cause reduced GTP-mediated inhibition of the enzyme [42]. Missense mutations in exons 11 and 12, corresponding to the allosteric domain, result for instance in Lys-450-Glu [42], Ser-445-Leu, Gly-446-Ser, Gly-446-Asp, Ser-448-Pro, His-454-Tyr modifications [20]. Other missense mutations located in *GLUD1* exons 6 and 7, corresponding to the catalytic domain of the enzyme, consist in Ser-217-Cys [43], Arg-221-Cys [44], Arg-265-Thr [43], Tyr-266-Cys [43], Arg-266-Lys [45], Arg-269-His [44], Arg-269-Cys [43] and Glu-296-Ala [45] substitutions that are also responsible for the hyperinsulinism/hyperammoanemia syndrome [44,45]. Surprisingly, mutations in exons 6 and 7 result in diminished inhibitory effect of GTP on GDH activity, similar to effects of mutations in exons 11 and 12. This suggests that amino acid substitutions in the catalytic domain could result in severely altered tertiary structure of the enzyme indirectly affecting the GTP binding site [44]. Finally, activating missense mutation in exon 10, responsible for an Asn-410-Thr in GDH protein, has been identified and is located outside of the allosteric domain [21].

3. GDH function in specific tissues

Although GDH enzyme catalyzes the same reaction in every tissue, its function regarding metabolic homeostasis varies greatly according to specific organs. We will illustrate such diversity through three tissues controlling whole body metabolism at different levels; i.e. insulin secretion in pancreatic β-cells, ammonia metabolism in hepatic cells, and glutamate/glutamine cycling in the central nervous system.

3.1. GDH and pancreatic β-cell function

Pancreatic β-cells produce the hormone insulin and insulin action on target tissues maintains glucose homeostasis. Upon nutrient stimulation, elevation of cytosolic Ca^{2+} in the β-cell is the primary and necessary signal for insulin exocytosis [46]. Then, increasing the magnitude of the secretory response requires amplification of the Ca^{2+} signal involving metabolism-derived additive factors [18]. Importance of GDH as a key enzyme in the control of insulin secretion has been recognized long ago [16]. However, the complete detailed function of GDH in β-cells remains to be determined. Specifically, GDH might play a role in glucose-induced amplifying pathway through generation of glutamate [4,47]. GDH is also an amino acid sensor triggering insulin release upon glutamine stimulation in conditions of GDH allosteric activation [48–50]. These two modes of action each requires opposite directions of GDH reactions. GDH preferential flux direction would be either anaplerotic forming α-ketoglutarate from glutamate or, conversely, cataplerotic generating glutamate at the expense of α-ketoglutarate. Temporally, these two actions are not mutually exclusive, as specific metabolic states of the cell might dictate one or the other direction. During glucose stimulation, elevation of mitochondrial NADH to NAD^+ ratio should favour NADH-dependent reductive transamination of α-ketoglutarate to glutamate. Indeed, such metabolic conditions result in saturated electron transport chain activity [51]. Then, GDH is the only alternative enzyme to complex I activity for NADH reoxidation to NAD^+. NMR spectroscopy studies performed on insulinoma cells revealed that under glucose stimulation glutamate is generated rather than being consumed [52] and that pyruvate dehydrogenase plus pyruvate carboxylase act as the anaplerotic enzymes rather than the GDH [53]. Therefore, the flux direction of GDH might depend on redox and energy state of mitochondria, as well as on provision of specific substrates determining the balance of cataplerosis versus anaplerosis.

Of interest, GDH was investigated in β-cells mostly by means of increased activity of the enzyme. Numerous studies have used the GDH allosteric activator L-leucine or its non-metabolized analogue beta-2-aminobicyclo[2.2.1]heptane-2-carboxylic acid (BCH) to question the role of GDH in the control of insulin secretion [16,48,49,54,55]. Our group also increased GDH activity by means of overexpression, an approach that we combined with allosteric activation of the enzyme [56].

As described above, another line of studies in β-cells looked at activating mutations of GDH that have been associated with a hyperinsulinism syndrome [20,21]. Reduced GTP-mediated inhibition of the enzyme was associated with most of GDH mutations linked to the hyperinsulinism syndrome [42]. β-cells forced to express such mutations became glutamine responsive in terms of insulin secretion, both in cell and mouse models [50,57]. Unlike glucose, glutamine is not efficiently catabolised to the end-product CO_2 in β-cells [55,58]. However, glutamine complete oxidation can be prompted by allosteric activation of GDH, an effect correlating with stimulation of insulin secretion [48,55]. Alternatively, we have shown that overexpression of GDH in isolated islets promotes glutamine metabolism associated with insulin secretion [56]. Taken together, these gain-of-function approaches demonstrate that glutamine can be turned into a secretagogue under conditions of increased GDH activity, although glucose stimulated insulin secretion is not, or very modestly, modified [50,54,56,57].

In only limited number of studies, GDH activity was reduced in pancreatic islets by the use of inhibitors for which specificity is questionable [17,19,59]. Upon glucose stimulation, GDH inhibition results in both lower insulin release and cellular glutamate levels [59,60]. Green tea polyphenols inhibit GDH and reduce insulin release when islets are stimulated with glutamine plus BCH, although not upon glucose stimulation [19]. Recently, it has been shown that the mammalian Sir2 homolog SIRT4 acts in the mitochondria of pancreatic β-cells to repress the activity of GDH through ADP-ribosylation [36,61]. SIRT4 downregulates GDH activity and thereby modulates insulin secretion [35,36]. In particular, we have shown that glucose stimulated insulin secretion is increased when SIRT4 is downregulated and decreased when overexpressed [35]. Antisense approach was also used in our laboratory to lower GDH expression in rat clonal INS-1E β-cells, resulting in reduced GDH activity and inhibition of glucose evoked insulin release [18].

3.2. GDH and hepatic function

The liver is the centre of nitrogen metabolism in the organism. Nitrogen is transported from skeletal muscle essentially as glutamine and alanine. In the liver, hepatic glutamate pathway is involved for metabolism of most amino acids through transamination or GDH activity. Glutamate metabolism is strongly compartmentalized [62]. Urea synthesis takes place in periportal hepatocytes where glutamine is deaminated by glutaminase, thereby generating ammonia and glutamate. Transaminases, such as alanine aminotransferase, are also more abundant in the periportal region. Then, glutamate is mainly taken up by the small hepatocytic population in the perivenous hepatocytes where GDH and glutamine synthetase are abundantly expressed [63]. Hepatic GDH has a rather high activity and can be

involved in both glutamine utilization and synthesis. This suggests that net flux can be in either direction, depending on the provision or clearance of substrates and products along with allosteric control. For instance, it has been suggested that a rise in hepatic glutamate concentrations enhances ureagenesis in response to protein feeding [64]. By transamination reactions producing α-ketoglutarate, glutamate can transfer its amino group to either oxaloacetate or pyruvate, thereby generating aspartate or alanine, respectively. Alternatively, glutamate can be deaminated and oxidized via GDH to yield α-ketoglutarate (also named 2-oxoglutarate). Next, α-ketoglutarate is further oxidized by the TCA cycle, providing energy in the form of ATP or utilized for gluconeogenesis [65]. The first step of urea synthesis is controlled by *N*-acetylglutamate, a glutamate metabolite that activates the mitochondrial carbamoyl phosphate synthetase [66]. Therefore, GDH function is essential to preserve urea generation and to control ammonia levels. The importance of GDH activity is witnessed by the severity of disorders where GDH function is compromised, such as hyperinsulinism/hyperammonaemia syndrome and N-acetylglutamate synthase deficiency.

3.3. GDH and brain function

Mitochondrial function in the brain is closely associated with glutamate metabolism as this amino acid is the main excitatory neurotransmitter in the central nervous system [67]. In particular, mitochondria regulate energetic status of the brain, utilization of metabolic substrates, and detoxification associated with excess of neurotransmitters.

Glutamate is released by activated synapses and then depolarizes target neurons through specific receptors [68,69]. Glutamate is also the precursor of GABA in neurons and of glutamine in astrocytes [70,71]. During the synaptic glutaminergic transmission, extracellular levels of glutamate rise dramatically [72]. Then, glutamate is promptly removed in order to maintain low concentrations in the extracellular space (below 3 μM) to avoid excessive stimulation of its receptors that would result in dysfunction of downstream signaling system, i.e. excitotoxicity effects [73,74]. Intersynaptic glutamate clearance is mostly operated by astrocytes, in particular by their specialized transmembrane transporters GLT1/EAAT2 and GLAST/EAAT1 [75]. GLT1 and GLAST are expressed mainly in astrocytes [76] and take up glutamate through a Na^+-dependent high affinity system [77,78]. Astrocytic internalization of glutamate not only participates to detoxification of the extracellular space, but also supplies energy to brain cells. Neurons and astrocytes are metabolically coupled and tightly related in the regulation of energy balance of the brain. Astrocytes take up glutamate essentially via Na^+-glutamate co-transporter. When extracellular glutamate is elevated, glutamate-induced Na^+ influx is paralleled by Na^+ extrusion through energy dependent Na^+/K^+ ATPase [73]. Once internalized inside the astrocyte glutamate is transformed into glutamine by the enzyme glutamine synthase [79]. Glutamine is then transported to neurons, transformed back into glutamate by glutaminase, and packed into secretory vesicles to be released as neurotransmitter [80]. The alternative fate of astrocytic glutamate is the oxidative transamination into α-ketoglutarate and complete oxidation via the TCA cycle.

Upon neurotransmission, glutamate stimulates its own oxidation in order to compensate for increased energetic demand. The ratio between glutamate/glutamine cycling and oxidative metabolism of glutamate varies according to neuronal activity, the rate of oxidized glutamate rising from 10% to 50% during stimulation [81]. Glutamine is a precursor for glutamate used either as a neurotransmitter in neurons or as a metabolite providing energy to astrocytes via glutaminase action located in the inner mitochondrial membrane [82]. Overall, glutamate is an important source of energy for glial cells [83].

In the brain, GDH is highly expressed in astrocytes [84]. This ensures efficient clearance and catabolism of glutamate released from neurons [14,85]. As described above, human GDH exists both as ubiquitous enzyme (hGDH1) encoded by the *GLUD1* gene and as brain and testicular tissue-specific isoform (hGDH2) encoded by *GLUD2* [38]. The *GLUD2* gene encodes a GDH that is resistant to GTP-mediated inhibition [86]. Such *GLUD2* encoded GDH might confer to astrocytes higher capacity of glutamate catabolism following glutamate release as neurotransmitter [41], thereby preventing glutamate induced neurotoxicity [87]. In rodents, *GLUD1* is the only isoform of GDH [41].

GDH is primarily expressed in the mitochondrial matrix of astrocytes and to a lesser extent in neurons [88]. Under basal conditions, astrocytes metabolize glutamate via glutamine synthetase rather than via GDH, thereby favoring glutamine formation [89]. The amount of glutamate being oxidized through the TCA cycle increases dramatically when extracellular glutamate is raised to concentrations reaching 0.5 mM [81]. Under such conditions requiring energy supply, astrocytes metabolize glutamate primarily via GDH, although in neurons glutamate is mostly processed by aspartate aminotransferase. Complete oxidation of glutamate occurs in the TCA cycle, where glutamate enters as α-ketoglutarate after oxidative deamination catalyzed by GDH [90]. Oxidative catabolism of glutamate to CO_2 produces energy in the form of ATP. Differences in glutamate handling between neurons and astrocytes provide functional example of energetic compartmentalization. This way, GDH oxidizes endogenous glutamine in neurons and exogenous glutamine in astrocytes [91].

4. Mitochondrial glutamate carriers

The transport of glutamate across the inner mitochondrial membrane is mediated by two identified transporters (Fig. 2), i.e. the aspartate–glutamate exchanger and the glutamate carrier [92–94]. Both proteins belong to the mitochondrial solute carrier protein family, which comprises over 45 members, among them the well known uncoupling proteins and the ADP/ATP carriers [95–99]. Unique properties have been ascribed to the solute carriers such as the tripartite structure and the six transmembrane hydropathy profile, which has facilitated the identification of other potential members [97–99]. The major drawback for the functional characterization of these carriers may be accounted for by the minute expression of these proteins [99].

As inferred from their names the glutamate carrier (GC) as well as the aspartate–glutamate carrier (AGC) mediate the transport of glutamate, along with a proton for the former, or in exchange with aspartate for the latter [93,94]. The GC family is electroneutral, whereas AGC is electrogenic. AGC requires concentration gradient of solutes and/or electrochemical potential generated across the inner mitochondrial membrane as a driving force [100]. The other major difference between the two carriers is that the AGC has a bipartite structure, i.e. the amino termini contain four EF Ca^{2+} binding sites that are facing the external side of the inner mitochondrial membrane, whereas the carboxyl end is characteristic of mitochondrial solute carriers [101]. This suggests the possible implication of this carrier as a Ca^{2+} sensor. GC on the other hand has the typical profile of solute carriers [102]. Several studies focused on the physiological and pathological relevance of both carriers that have been recently reviewed in details [103].

4.1. Aspartate–glutamate carrier (AGC)

The aspartate–glutamate isoforms 1 and 2 are also commonly referred to as Aralar1 and citrin carriers [103]. Aralar1 derives from the contraction of author's name (ARAceli del Arco) with reference to the fact that the protein is longer in size when compared to other solute carriers (hyperLARgo) [101]. The citrin carrier relates to the implication of AGC2 with human citrin deficiency [104]. Several functions have been ascribed to aspartate–glutamate carriers. In particular, they constitute part of the malate–aspartate shuttle [103,105]. This shuttle mediates the transfer of reducing equivalents from cytosolic NADH into mitochondria where it activates the electron transport chain. This,

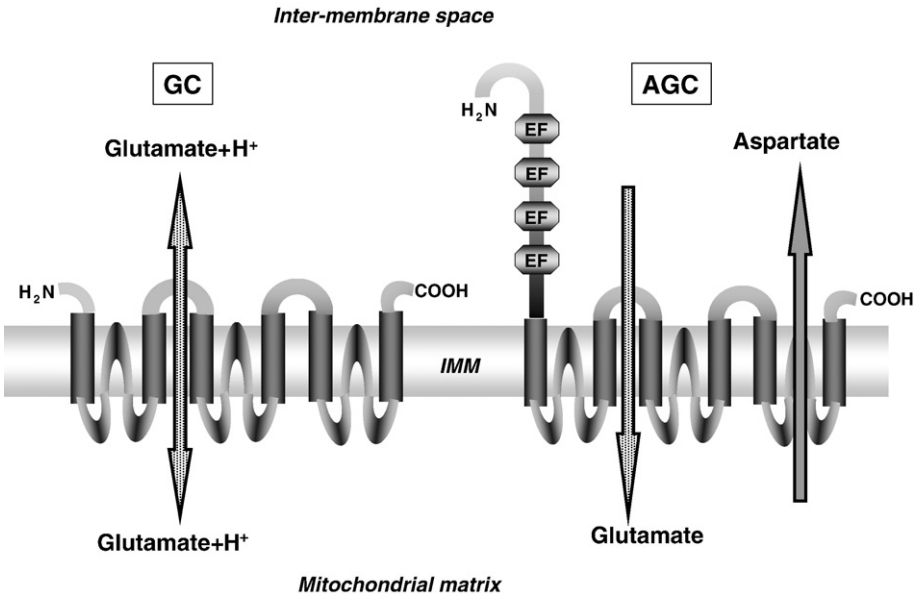

Fig. 2. Simplified scheme of glutamate carrier (GC) and aspartate–glutamate carrier (AGC). Both carriers span the inner mitochondrial membrane (IMM) six times with three long hydrophilic loops. AGC has a bipartite structure, with the C-terminal domain that is similar to GC and the N-terminal domain harbouring EF-Ca^{2+} binding motifs. GC mediates the transport of glutamate along with a proton, whereas AGC carries glutamate into the mitochondrial matrix in exchange to aspartate.

in turn, leads to ATP generation, rendering AGCs major contributors for the event of oxidative phoshorylation [105–107].

Both AGC1 and AGC2 are differentially expressed in specific tissues, although both are found in heart and kidney. Aralar1 is expressed in excitable tissues, such as the brain and skeletal muscle [101], whereas the citrin carrier is found predominantly in the liver but also in the small intestine [108,109]. These carriers are involved in a number of metabolic pathways, including gluconeogenesis, ureogenesis and the generation of proteins and amino acids [105,110], accounting for its expression in the aforementioned tissues to accommodate high metabolic demands. In the prospect of clarifying the role of AGCs, knockout mice for both Aralar and citrin were generated.

Aralar1 deficient mice exhibit severe growth defects, limited lifespan and impairment of motor coordination, as well as deficits in myelination. In addition, the lack of Aralar1 accounted for the absence of malate–aspartate shuttle activity in both heart and brain as expected [110].

Since AGCs are Ca^{2+} sensors, they might be implicated in Ca^{2+} signalling. Accordingly, Ca^{2+} mobilising agonists are able to increase mitochondrial ATP levels, suggesting that the Ca^{2+} binding site of AGCs is crucial for the control of malate–aspartate shuttle activity [111,112]. Our laboratory pointed out the importance of Aralar1 towards glucose stimulated insulin secretion in the context of pancreatic β-cells [107]. Adenoviral mediated over-expression of Aralar1 ameliorates coupling between glycolysis and mitochondrial activation. In particular, increased expression of Aralar1 potentiates metabolism secretion coupling by increasing NADH generation, ATP levels, glucose oxidation, and insulin secretion, along with reduced lactate production [107]; thereby stressing the importance of the malate–aspartate shuttle for β-cell function [107,113].

Human citrin deficiency encompasses both adult onset type II citrullinemia (CTLN2) and neonatal intrahepatic cholestasis (NICCD). CTLN2 disorder is characterized by the accumulation of citrin in the body due to a deficiency in the enzyme argininosuccinate synthase [114]. Clinically, CTLN2 patients display a wide variety of neuropsychiatric symptoms associated with hyperammonia, which may lead to sudden death between the ages of 20 to 40 as a result of brain edema [104,115]. It was shown that liver transplantation may effectively eradicate all symptoms associated with CTLN2, suggesting that argininosuccinate synthetase deficiency is liver specific [115]. NICCD on the other hand, has less severe clinical symptoms, restricted to the first few months after birth. Typically, patients suffer from multiple metabolic abnormalities, such as aminoacidemias, galactosemia, and hypoproteinemia [115].

AGC2 knockout mouse model was generated as a representative model of CTLN2 pathology [116]. These mice showed a significant decrease in aspartate efflux from liver mitochondria when compared to the wild type mice. Moreover, liver perfusion revealed marked decrease of ureogenesis from ammonia and gluconeogenesis from lactate. Despite the deficits in AGC-dependent metabolic pathways, the knockout mice failed to display physiological factors that are characteristic of CTLN2 [116].

As inferred from the knockout mice models that were previously described, these studies demonstrate that there is not a defined correlation between a defective gene and its pathology. The complexity is further enhanced by environmental factors, which may account for strong ethnic biases, such as the ones observed for CTLN2 in Asian population [117–119]. Despite the apparent complexities linked to pathogenesis onset, the use of knockout mouse may be informative although one should be aware of compensatory pathways, which may alleviate or totally abrogate the expected phenotype.

4.2. Glutamate carrier (GC)

The glutamate carrier was first reported in the early seventies by Meijer et al. and its identification was supported by the evidence of mitochondria swelling in the presence of ammonium glutamate [94]. It was found that GCs play a crucial role in ureogenesis, since the provision of glutamate for the production of NH_3, mediated by the enzyme GDH, is exclusively derived from glutamate carriers. Glutamate derived from the aspartate–glutamate carriers is transaminated with intramitochondrial oxaloacetate to form aspartate, thus not available for GDH [120].

Regarding glutamate transport in liver mitochondria, it is relatively slow under in vivo conditions, although correlating with the rate of urea production [121]. A study investigating in the liver directionality of glutamate transport across the inner mitochondrial membrane demonstrated preferential export from the mitochondrion to the cytosol [97,122]. However, it should be stressed that directionality of glutamate transport is dependent on mitochondrial pH, since glutamate efflux is determined by matrix acidification and media alkalinisation [122]. The pH dependency of glutamate transport may

account for functional differences of glutamate according to tissues. Thus, with respect to glutamate transport rate, it was found to be higher in the brain, slower in the liver, and more so in the kidney when compared to other solute carriers, which correlates with the importance of glutamate in the brain and its implication in liver nitrogen metabolism [121,123].

Currently, very little is known on glutamate carriers. Two isoforms have been identified, GC1 and GC2, which are highly similar in amino acid sequences [102]. What differentiates both isoforms is the level of expression in various human tissues. Thus, although both isoforms are equally expressed in the brain, GC1 is highly expressed in the pancreas and the liver. This correlates with differences in their kinetic parameters, since GC1 has a higher Km value when compared to GC2 [102]. Of interest, the Km value of GC1 correlates with the Km value for glutamate uptake measured in liver and kidney mitochondria [120–123], thereby suggesting that in both organs glutamate uptake is mediated essentially through GC1. Directionality of glutamate transport might be dependent on the energized state of mitochondria. Therefore, one cannot safely conclude that glutamate is preferentially exported as was suggested previously, since experiments performed on reconstituted liposomes revealed that both directions are plausible [102].

As opposed to AGCs, there are no human pathologies associated with GC1 to date, except for a recent correlation between GC1 mutation and neonatal myoclonic epilepsy [124]. This might change in the future, since further characterization of GCs would clarify the role of glutamate in various tissues.

5. Conclusion

Glutamate pathways are tightly controlled by mitochondrial metabolism. Although enzymes and pathways of the mitochondrial matrix have been studied quite extensively, the regulation of mitochondrial membrane carriers is poorly characterized. Once established the basis of molecular properties of such enzymes and transporters, we will need to acquire knowledge about tissue specificities. Recent advances show that it is inappropriate to extrapolate regulation models acquired from one cell model to another, as every tissue uses glutamate for specific functions. Very little is known about molecular mechanisms responsible for tissue specificities. For instance, expression of different isoforms of glutamate carriers might contribute to tissue specificity. Regarding GDH, flux direction depends on metabolic parameters such as substrate availability, redox and energy state of mitochondria. These parameters may be tissue specific as described above. At the post-translational level, new modes of regulations have been described these recent years. Indeed, ADP-ribosylation of GDH mediated by SIRT4 offers another regulatory mechanism that might be tissue specific, pending different levels of *SIRT4* expression. Noteworthy, SIRT4 is expressed at low levels in pancreatic acini, whereas at high levels in pancreatic islets [35]. This newly identified mode of regulation certainly deserves further investigations to better integrate molecular and cellular glutamate pathways into metabolic homeostasis at the organism level.

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