BBA - Molecular Cell Research 1870 (2023) 119468

Contents lists available at ScienceDirect

BBA - Molecular Cell Research

journal homepage: www.elsevier.com/locate/bbamcr

Regulation of neuronal energy metabolism by calcium: Role of MCU and Aralar/malate-aspartate shuttle

Araceli del Arco [a,b,c,d,e,*], Luis González-Moreno [a,b,c], Irene Pérez-Liébana [a,b,c], Inés Juaristi [a,b,c], Paloma González-Sánchez [a,b,c], Laura Contreras [a,b,c], Beatriz Pardo [a,b,c], Jorgina Satrústegui [a,b,c]

[a] Departamento de Biología Molecular, Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas-Universidad Autónoma de Madrid (CSIC-UAM), Madrid, Spain
[b] Instituto Universitario de Biología Molecular (UAM), Madrid, Spain
[c] Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD), Madrid, Spain
[d] Facultad de Ciencias Ambientales y Bioquímica, Universidad de Castilla la Mancha, Toledo, Spain
[e] Centro Regional de Investigaciones Biomédicas, Unidad Asociada de Biomedicina UCLM-CSIC, Toledo, Spain

ARTICLE INFO

Keywords:
Calcium signaling
Respiration
Redox shuttles
Neuronal metabolism
Neuronal activity
Mitochondrial carriers
Bioenergetic

ABSTRACT

Calcium is a major regulator of cellular metabolism. Calcium controls mitochondrial respiration, and calcium signaling is used to meet cellular energetic demands through energy production in the organelle. Although it has been widely assumed that Ca^{2+}-actions require its uptake by mitochondrial calcium uniporter (MCU), alternative pathways modulated by cytosolic Ca^{2+} have been recently proposed. Recent findings have indicated a role for cytosolic Ca^{2+} signals acting on mitochondrial NADH shuttles in the control of cellular metabolism in neurons using glucose as fuel. It has been demonstrated that AGC1/Aralar, the component of the malate/aspartate shuttle (MAS) regulated by cytosolic Ca^{2+}, participates in the maintenance of basal respiration exerted through Ca^{2+}-fluxes between ER and mitochondria, whereas mitochondrial Ca^{2+}-uptake by MCU does not contribute. Aralar/MAS pathway, activated by small cytosolic Ca^{2+} signals, provides in fact substrates, redox equivalents and pyruvate, fueling respiration. Upon activation and increases in workload, neurons upregulate OxPhos, cytosolic pyruvate production and glycolysis, together with glucose uptake, in a Ca^{2+}-dependent way, and part of this upregulation is via Ca^{2+} signaling. Both MCU and Aralar/MAS contribute to OxPhos upregulation, Aralar/MAS playing a major role, especially at small and submaximal workloads. Ca^{2+} activation of Aralar/MAS, by increasing cytosolic NAD^+/NADH provides Ca^{2+}-dependent increases in glycolysis and cytosolic pyruvate production priming respiration as a feed-forward mechanism in response to workload. Thus, except for glucose uptake, these processes are dependent on Aralar/MAS, whereas MCU is the relevant target for Ca^{2+} signaling when MAS is bypassed, by using pyruvate or β-hydroxybutyrate as substrates.

1. Introduction

The regulation by calcium of mitochondrial energy metabolism has been known for a long time [1–3]. Ca^{2+} is required to sustain cellular respiration in resting conditions [4] and Ca^{2+} signals mediate its regulation in different situations in which cells need to increase ATP synthesis. During activation, neurons, as other excitable cells, suffer large fluctuations of Ca^{2+} and other ions, that elicit ATP-consuming intracellular processes, and in turn Ca^{2+} transients stimulate energy production to meet ATP demand with ATP production [5]. Ca^{2+} uptake in mitochondrial matrix through the mitochondrial calcium uniporter complex (MCUC) activates the dehydrogenases of Krebs' cycle and provides reducing equivalents (NADH) to feed the electron transport chain (ETC) and enhance ATP generation [1–3]. On the other side, Ca^{2+} may modulate respiration, ATP production, by increasing metabolite supply by the action of the Ca^{2+}-regulated mitochondrial carriers (CaMCs) which are activated by cytosolic Ca^{2+} (reviewed by [6]). CaMCs family comprises two members, the aspartate/glutamate carriers (AGCs) and the ATP-Mg^{2+}/phosphate carriers. Both have a transport domain inserted into the membrane and long N-terminal extensions which contain EF-hand Ca^{2+}-binding motifs facing the intermembrane space ([7,8]; reviewed in [6,9]). Although Mg^{2+}-binding cannot be ruled

* Corresponding author at: Facultad de Ciencias Ambientales y Bioquímica, Universidad de Castilla la Mancha, Toledo, Spain.
E-mail addresses: adelarco@cbm.csic.es, araceli.arco@uclm.es (A. del Arco).

https://doi.org/10.1016/j.bbamcr.2023.119468
Received 13 February 2023; Received in revised form 17 March 2023; Accepted 21 March 2023
Available online 28 March 2023

out, CaMCs EF-hands display features of Ca^{2+}-binding EF-hand loops as the presence of glutamate at the loop's C-terminus [7,8] which allow a preferential coordination of Ca^{2+} rather than Mg^{2+} [10]. AGCs are the Ca^{2+}-regulated components of the malate/aspartate shuttle (MAS) involved in NADH shuttling from the cytosol to mitochondria [11–14].

In this review, we will summarize recent advances on the contribution of the AGC/MAS pathway, modulated by cytosolic Ca^{2+} signals, and mitochondrial Ca^{2+} uptake by the MCU pathway in the regulation of mitochondrial respiration in neurons regarding: i) the control of basal respiration by ER-to-mitochondria Ca^{2+} fluxes, ii) the regulation by Ca^{2+} of mitochondrial respiration and neuronal metabolism in response to agonists.

2. Regulation by Ca^{2+} of basal respiration in neurons: RyRs and the malate/aspartate shuttle participate in the regulation of basal respiration by Ca^{2+} in neurons using glucose as fuel

An endoplasmic/sarco reticulum (ER/SR) to mitochondria constitutive low-level Ca^{2+} flow has been demonstrated to be important in regulating basal respiration in cell lines [4,15–18] and in different tissue-derived cells [18–21]. Regulation of basal respiration by ER-derived Ca^{2+} was assumed to be due to Ca^{2+} entry in mitochondria and activation of Krebs' cycle dehydrogenases that supply reducing equivalents to the electron transport chain (ETC) [1,3]. Since the Inositol 1,4,5-trisphosphate receptors (InsP3Rs) are in close apposition to mitochondria these Ca^{2+} channels were proposed as main contributors of ER/SR-derived Ca^{2+} modulating mitochondrial function [22]. DT40 B lymphocytes, and other cell lines as HEK-293 or 143B, lacking all three InsP3R isoforms showed a reduction in the basal oxygen consumption rate (OCR) that was recovered in the presence of the Ca^{2+} ionophore ionomycin, suggesting that Ca^{2+} availability was limiting for normal oxidative phosphorylation (OxPhos) [4,15,23]. Accordingly, treatment with Xestospongin B (XeB) a specific InsP3R inhibitor [24] dropped cellular respiration in HEK-293 and lymphoblastic leukemia cells [4,15,25].

In neurons, however, a role for ryanodine receptors (RyRs), and not InsP3R, has recently been recognized as source of ER Ca^{2+} efflux [18,21]. On the one side, 3-dmXeB, a XeB analog, did not have any effect on basal OCR in cortical neurons [18], showing that Ca^{2+} efflux from the ER through InsP3R does not play any relevant role in the control of basal respiration in neurons. On the other side, a drop in the cellular ATP/ADP ratio, coupled to decreased cytosolic Ca^{2+} transients, was observed in dopaminergic neurons after the inhibition of RyRs with DHBP (1,1′-diheptyl-4,4′-bipyridinium dibromide) [21]. Similarly, knockdown (KD) of *RyR2*, the main RyR isoform in the brain [26], using specific shRNAs or the presence of RyRs inhibitors such as ryanodine or dantrolene, caused a significant drop of basal and oligomycin-sensitive respiration in cortical neurons [18]. Furthermore, an increase in the AMPK phosphorylation status, an autophagy marker associated with the failure to maintain ER to mitochondria Ca^{2+} flux [4,27], was observed in *RyR2*-KD neurons [18].

A priori these findings were unexpected since InsP3R1 is abundant in brain neurons specially in cerebellum [28]. Nevertheless, RyRs are also close to mitochondria in neurons [29], facilitate a rapid Ca^{2+} transfer from ER to the matrix [30] and participate in mitochondria-associated ER membrane (MAM) formation in brain [31]. Furthermore, this role for RyRs is not restricted to neurons, as RyRs along with InsP3Rs have been involved in the control of basal OCR in skeletal muscle [19]. Moreover, both ER Ca^{2+} efflux pathways might be functionally equivalent, as it has been suggested by the fact that they were reciprocally regulated [32]. Thus, in muscle fibers *RyR1* silencing led to a significant increase in basal respiration, which was prevented by XeB [32].

It has widely been assumed that the action of ER Ca^{2+} leak on OxPhos requires its entry into the organelle via the mitochondrial calcium uniporter (MCU) [2]. However, the extremely mild phenotype in the MCU-KO mouse [33] and the absence of respiratory deficits in MCU-deficient models has called this notion in question [17,18,21,34–39]. Recent findings obtained employing different approaches, using cells derived from MCU null mice or by AAV-mediated MCU KD, showed that MCU deficiency had no influence on basal respiration in cortical neurons when glucose was used as a fuel [18,37,38]. In this condition, there were no significant differences in ATP-linked [18,37,38] or in maximal respiration [18,37,38]. Similarly, the contribution of mitochondria and mitochondrial OxPhos to the maintenance of the cytosolic ATP/ADP ratio and bioenergetic status remained unchanged in dopaminergic neurons from substantia nigra pars compacta (SNc) lacking the MCU compared with WTs [21].

For some MCU-deficient models such as the global MCU-KO mouse [33] it was suggested that the lack of phenotype was due to the existence of alternative mitochondrial Ca^{2+} transporters [34,40], or due to compensatory mechanisms that masked its true role [20,41–44]. However, it is unlikely that compensatory mechanisms are involved in the absence of effect on respiration in neurons from MCU-KO mouse since similar results were observed after acute MCU KD in neurons using shRNAs [18,21,38]. Furthermore, no substantial variations in respiration were obtained in brain mitochondria from MCU-KO [39]. The lack of compensatory mechanisms agrees with the unaltered expression of genes associated with mitochondrial functions detected in neurons from hippocampus [45] or SNc dopaminergic area [21] of MCU-deficient mice. Accordingly, MCU-KO dopaminergic neurons did not show alterations in mitochondrial morphology or density, and these mice show a normal behavior in open field test, gait, or fine motor skills [21].

Interestingly, the lack of effect of MCU deficiency on cell respiration is not restricted to neurons; respiratory alterations were not observed in intact myofibers isolated from skeletal muscle-specific MCU KO mice when using glucose [46] or other cell types like mouse embryonic fibroblasts (MEFs), T-cells or brown adipocytes lacking MCU [33,44,47]. Similarly, basal respiration did not show dependence on MCU activity when Ca^{2+} fluxes from ER to mitochondria were attenuated by short-term removal of extracellular Ca^{2+} in an endothelial-derived cell line [17]. Furthermore, in the absence of EMRE, an essential regulator of MCU activity, ATP levels and oxygen consumption remained virtually unaffected in EMRE KO MEFs [48,49]. In fibroblasts from patients with EMRE variants causing aberrant mitochondrial Ca^{2+} uptake, basal and ATP-linked respiration also remained unaltered [50]. Likewise, in cells with silenced *MICU1*, a MCU gatekeeper required to facilitate its activation at high cytosolic [Ca^{2+}] [51], basal OCR and other respiratory parameters were fully preserved [52].

Therefore, if Ca^{2+} uptake through MCU is dispensable, as part of the ER to mitochondria Ca^{2+} flow, to maintain basal respiration in neurons using glucose, and probably in other cell types, how is Ca^{2+} regulation of respiration achieved? Reducing equivalents from cytosolic NADH can fuel OxPhos after transference to mitochondria through Ca^{2+} sensitive NADH shuttling systems, the malate/aspartate and glycerol-3-phosphate shuttles. These shuttles have now been proposed as major determinants of activity-dependent mitochondrial ATP production in neurons [18,21,39,53–55].

Although their dependence on Ca^{2+} is well known, the participation of mitochondrial NADH shuttles in the transduction of cytosolic Ca^{2+} signals to mitochondria, as alternative to Ca^{2+} uptake by MCU, is not yet fully recognized (reviewed in [6]). However, in the last years, results obtained from different neuronal models devoid of malate/aspartate shuttle (MAS) components [17,18,39,54–56] or using specific inhibitors of MAS or the glycerol-3-phosphate shuttle (G3PS) [21,53] have revealed their involvement in the regulation of OxPhos either in basal conditions (non-stimulated) or under different stimuli (see below). In neurons, it was shown that in resting conditions, using glucose as fuel at physiological concentrations (2.5–5 mM), mitochondrial respiration was dependent on MAS [54–56]. In these conditions, cortical neurons deficient for the aspartate/glutamate isoform AGC1/Aralar, the Ca^{2+}-sensitive component of MAS, showed about a 40 % drop in basal respiration [54,56,57]. Similarly, knockdown of *AGC2/citrin*, the major

AGC isoform in non-excitable tissues [8], in endothelial cells caused a reduction in basal levels of NADH and ATP in mitochondria, as well as in maximal NADH production, similar to that produced by lowering Ca^{2+} levels in the mitochondrial intermembrane space [17]. As Ca^{2+}-binding domains of AGCs face the intermembrane space which connects to the cytosol, cytosolic Ca^{2+} signals modulate MAS activity which may act as a source of NADH for the mitochondrial matrix even when cytosolic Ca^{2+} signals are below the activation range of MCU [11,14].

In neurons using glucose the production of NADH in the mitochondria is preferentially fueled by pyruvate, produced by glycolysis; pyruvate can also be produced from lactate re-oxidation by lactate dehydrogenase (LDH), the two processes linked to the action of redox shuttles. Glycolysis can only progress if there is sufficient supply of NAD^+. The oxidation of glucose requires NAD^+ (2 molecules for each glucose) that becomes NADH in the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) reaction. Therefore, to sustain glycolysis, NAD^+ needs to be continuously regenerated either by LDH with conversion of pyruvate to lactate, or by NADH shuttle systems of which MAS is the prevalent in neurons [58]. AGC1/Aralar, as component of MAS (Aralar/MAS), will increase reducing equivalents supply from the cytosol and cause an enhancement of OxPhos rates acting similar to a "gas pedal" pushing pyruvate away from lactate and into OxPhos, a process regulated by cytosolic Ca^{2+} [14,18,39,54,55,57,59–62]. Indeed, oxygen consumption was reduced when pyruvate uptake by mitochondria was inhibited in hippocampal neurons [35]. Consistent with this role for MAS, the drop in basal respiration found in AGC1/Aralar-deficient neurons was totally rescued by the addition of exogenous pyruvate [18,54,55,57]. Furthermore, *AGC2/citrin* silencing reduced mitochondrial pyruvate supply, determined in vivo using a mitochondrial pyruvate sensor, mito-PyronicSF [63], whereas *MCU* silencing did not cause any effect [17]. Consequently, as MAS activity depends on cytosolic Ca^{2+}, pyruvate uptake in mitochondria was reduced when extracellular Ca^{2+} is removed for a short period [17].

Therefore, in neurons, RyRs and Ca^{2+}-regulated MAS components contribute to the maintenance of local Ca^{2+}-fluxes between ER and mitochondria involved in the control of basal respiration whereas InsP3Rs and mitochondrial Ca^{2+}, i.e. that taken up through MCU complex, do not. Aralar/MAS, stimulated by cytosolic Ca^{2+}, will provide substrates, redox equivalents, and pyruvate, to feed respiration. However, while Aralar/MAS is required to maintain basal respiration in neurons using glucose, it may not be required with pyruvate or other fuels where the role of MCU remains unexplored. This may be also the case in cell lines and non-neuronal cells using high glucose concentrations, pyruvate and/or other nutrients in which redox shuttles and/or MCU may control basal respiration.

3. Ca^{2+}-induced workload and Ca^{2+} signaling: participation of Aralar/MAS and MCU pathways in the control of mitochondrial respiration under activation

Regarding the regulation of respiration in neurons, a very different scenario to consider is that involving large changes in workload, as a result of marked changes in the ionic composition of the cytosol which follow the opening of different types of ionic channels. To restore the resting state, neurons consume huge amounts of ATP used in pumping these ions out of the cell or into organelles. As one of these ions is Ca^{2+}, neurons can respond to the workload generated by ATP-consumption to restore Ca^{2+} and other ionic gradients, by increasing ATP production in mitochondria through the action of ATP synthase and/or, by increasing the rate of glycolysis [18,35,64]. A second mechanism to upregulate respiration independent of ATP demand, is via Ca^{2+} signaling. Indeed, elevations in cytosolic Ca^{2+} have been proposed to play a feed-forward role through their stimulatory influence on OxPhos [2,65] to accommodate the rapid acceleration in energy demand at the onset of intense activity. Ca^{2+} may potentiate the rate of OxPhos through the stimulation of Aralar/MAS and G3PS pathways [18,53] or, upon its uptake by MCU, through stimulation of the Ca^{2+}-sensitive dehydrogenases of the TCA cycle and ATP synthase. Telling apart the roles of Ca^{2+} in activation of OxPhos (ATP demand and/or Ca^{2+} signaling) has proved being quite a complex task and these roles have only been recently disentangled.

Studies performed on intact cortical neurons using glucose have demonstrated a role for Ca^{2+} signaling in the regulation of mitochondrial respiration in response to increased workloads caused by increases in cytosolic Ca^{2+} (carbachol) or Na^+ and Ca^{2+} (veratridine, high-K^+ depolarization, NMDA) concentrations [18,54,55,57]. Veratridine stimulated respiration in the presence of Ca^{2+} but much less in a Ca^{2+}-free medium which lowered the workload (estimated from rises in cytosolic Na^+ and Ca^{2+} levels) but paradoxically, resulted in a larger drop in cytosolic ATP. This paradox suggested that removal of a Ca^{2+} signaling component of the responses caused the blunted increase in respiration in Ca^{2+}-free media. Indeed, blocking Ca^{2+} signals (by incubation with the cytosolic Ca^{2+}-chelator BAPTA-AM which blocks Ca^{2+} signals while preserving the workload) was sufficient to reduce stimulated respiration in normal Ca^{2+} media. High K^+- or NMDA-stimulation caused a larger drop in ATP and a larger increase in respiration in the presence than in the absence of Ca^{2+} in the external medium, revealing a strong influence of Ca^{2+}-induced workload in the response. However, Ca^{2+} signaling also contributed to the response as incubations with BAPTA-AM in normal Ca^{2+} medium also blunted the increase in respiration [18,54].

Regarding the target of Ca^{2+} signaling, in every condition tested, Ca^{2+}-activation of Aralar/MAS was required to fully stimulate coupled respiration ([54]; revised in [57]). Aralar/MAS activation was required to promote pyruvate supply, since the addition of pyruvate abolished the differences in stimulation of OCR observed between AGC1/Aralar-deficient and WT neurons [18,54,55]. Therefore, Aralar/MAS is required to prime pyruvate entry in mitochondria as a step needed to activate respiration by Ca^{2+} in response to different workloads.

On the other hand, Perez-Liébana and coworkers have shown that in MCU-deficient neurons treated with carbachol (Cch), stimulation of respiration remained unaffected even though Cch-induced cytosolic Ca^{2+} oscillations failed to reach mitochondria in the absence of MCU [18]. MCU activity was also found dispensable to upregulate respiration in response to larger cytosolic Ca^{2+}-signals as those produced after the activation of NMDA receptors [18]. As expected for stimuli that lead to higher energetic demand, NMDA produced a greater stimulation in respiration which was largely AGC1/Aralar-dependent, and was unaffected when matrix Ca^{2+} uptake was hampered by MCU silencing [18]. These findings suggest that Ca^{2+}-modulated Aralar/MAS activity, but not MCU (at least at these stimulatory conditions), plays a critical role upregulating respiration in response to stimuli triggered by low and submaximal workloads. Therefore, in agreement with the Yellen and Gellerich's groups [35,39], MCU-induced Ca^{2+} entry in mitochondria and activation of mitochondrial dehydrogenases [3] is largely dispensable in the stimulation of respiration of neurons using glucose. However, under strong workloads such as those caused by veratridine in the presence of Ca^{2+}, a role for MCU is suggested as the stimulation of respiration was diminished but not blocked in the absence of Aralar/MAS [54].

Of note, as mentioned earlier for the regulation of basal respiration in neurons, the requirement for Aralar/MAS rather than MCU for OXPHOS stimulation is limited to the use of glucose as substrate, as replacing glucose by a mixture of lactate and pyruvate, or pyruvate alone, unveiled a strong influence of MCU in upregulation of ATP production by mitochondria [53,66].

Having stated these facts, the actual situation may not be so simple. Zampese et al. [21] using pacemaking SNc dopaminergic neurons in ex vivo brain slices, and a more physiological experimental approach have found conditions under which the roles of Ca^{2+}-induced workload and Ca^{2+} signaling were clearly dissected. In this study the contribution of these mechanisms to the energetic status of SNc dopaminergic neurons was determined by measuring changes in their repetitive spiking

behavior, and ATP/ADP ratio as readouts of ATP production. Inhibitors of Cav1 Ca^{2+} channels (isradipine) reduced cytosolic Ca^{2+} signals and caused a decrease in the ATP/ADP ratio indicating that Ca^{2+} was required to maintain the bioenergetics status through Ca^{2+} signaling. Disruption of MCU had no effects on the bioenergetics status of these neurons, even though the mitochondrial Ca^{2+} transient evoked during repetitive spiking were fully abolished. Similarly, aminooxyacetate acid (AOAA), which inhibits MAS, did not significantly change the spike rate. However, MAS inhibition in MCU-KO neurons induced a larger drop in cytosolic ATP/ADP ratio than in wild-type neuron, unmasking a role of Aralar/MAS in supporting OxPhos under these conditions. These results suggest that Ca^{2+} uptake by MCU and Ca^{2+}-stimulated Aralar/MAS activity could both work to provide ATP production by mitochondria necessary to support repetitive spiking [21]. Indeed, when glucose was replaced by β-hydroxybutyrate (β-OHB), which bypasses glycolysis and Aralar/MAS in Aralar-KO neurons [67] but fuels TCA cycle, the impact of MCU deletion in response to electrical stimulations become apparent. Under basal conditions, MCU KO neurons relying upon β-OHB show a spiking rate similar that of wild types, but upon a depolarization step were unable to sustain the elevated spiking rate observed in control neurons.

These intriguing results suggest that the MCU/dehydrogenases and Aralar/MAS pathways, the main targets of mitochondrial Ca^{2+} signaling in neurons may operate at the same time but with non-additive effects; with the MCU-pathway inhibiting MAS. The lack of additive effects of these two pathways, with MCU activity inhibiting MAS, was reported earlier [11,14,68]. It is based on the competition for their common substrate, α-ketoglutarate (α-KG), shared by MAS and the MCU-pathways (see Fig. 1) whose mitochondrial levels drop after Ca^{2+} activation of mitochondrial α-KGDH (also known as OGDH), causing a drop in the activity of the oxoglutarate/malate carrier (OGC) [11]. Inhibition of MAS by matrix Ca^{2+} was fully reversible once Ca^{2+} was removed from the matrix [11], was specific for brain mitochondria, and not observed in liver mitochondria [11]. Interestingly, Zampese et al. [21] found that MCU-KO neurons showed increased levels of α-KG, that explain the activation of MAS under these conditions.

The MCU and redox shuttles mechanisms of mitochondrial Ca^{2+} signaling might not simply be redundant mechanisms, but rather may be recruited in a substrate-dependent way (glucose/lactate or β-OHB) and enable that different intensities of stimulation elicit qualitatively distinct metabolic responses. In the case of those driven by Ca^{2+} signals, mitochondrial ATP production during larger workloads might be induced by a combined action of cytosolic and matrix Ca^{2+}, while smaller workloads might be transduced through an increased activity of NADH shuttles alone stimulated by cytosolic Ca^{2+}.

Because of their differences in relation to cytosolic Ca^{2+} concentration requirements, 100–300 nM for AGCs/MAS [14,59] and in the micromolar range for MCU [70], it is expected that, rather than simultaneously, Aralar/MAS and MCU pathways may be differentially, or sequentially, activated during Ca^{2+} transients. Recent findings obtained exposing cultured hippocampal neurons to electrical stimuli point in that direction [53]. Dhoundiyal et al. have evaluated cytosolic and mitochondrial Ca^{2+} transients and distinct bioenergetic parameters, mainly cytosolic ATP/ADP and NADH/NAD^+ ratios, in response to electrical field stimulation in the presence of specific inhibitors for MCU and NADH shuttles components. In these assays, it was observed that Aralar/MAS and MCU were differentially engaged in activity-dependent ATP production in mitochondria. Thus, MAS was operative at all intensities of electrical activity tested (2.5 and 10 Hz), whereas MCU contributed only at higher intensity (10 Hz), in those that produce larger cytosolic Ca^{2+} transients capable of reaching the matrix and to stimulate Ca^{2+}-sensitive dehydrogenases of the TCA cycle [53]. Likewise, by using specific inhibitors, Dhoundiyal et al., demonstrated that the glycerophosphate shuttle, a Ca^{2+}-activated NADH shuttle with low levels in neurons [6,56], could be functional in hippocampal neurons to maintain ATP supply in situations which MAS and MCU were unavailable [53].

Collectively, in situations of imposed or endogenous bursts of neuronal activity, Ca^{2+} exerts a clear role in upregulating OXPHOS either by increasing ATP demand and/or by Ca^{2+} signaling to mitochondria. In neurons using glucose, the targets of the Ca^{2+} signal in mitochondria are the Ca^{2+}-regulated redox shuttles (mainly Aralar/MAS but also G3PS) and MCU. The main role of Ca^{2+}-activation of Aralar/MAS is to provide pyruvate to mitochondria to prime OxPhos, a role bypassed by pyruvate supply while MCU activity stimulates mitochondrial dehydrogenases following the increase in matrix Ca^{2+}. Recruiting one or the other depends on the size of the Ca^{2+} signal (shuttles involved in small and MCU in large size Ca^{2+} signals) and overlaps among the two cause matrix Ca^{2+} inhibition of Aralar-MAS. This situation may be different when neurons use ketone bodies, pyruvate or other fuels which do not require Aralar/MAS, which will rely exclusively on MCU as target of mitochondrial Ca^{2+} signals.

4. Ca^{2+}-modulated Aralar/MAS activity provides a feed-forward mechanism to activate glycolysis

In response to an increase in workload, Ca^{2+} also activates glucose uptake and glycolysis together with respiration [18,71]. Ca^{2+} regulation of these processes could be also taken as a feed-forward mechanism upon initiation of the workload. Whether this occurs through a single mechanism or there are several Ca^{2+}-dependent steps that control the fate of glucose in neurons until its combustion in mitochondria is not yet well understood.

A Ca^{2+} dependent but Aralar/MAS independent [18] increase in glucose uptake upon synaptic activity or NMDA-stimulation has been found in both cerebellar and cortical neurons [18,64,72,73]. A translocation of glucose transporters, GLUT3/Slc2a3 [73] or GLUT4 [64], to the plasma membrane triggered by neuronal activity, via AMPK activation [64,72] has been proposed. As CaMKKβ is involved in AMPK activation in response to depolarization in neurons [74], it is possible that these mechanisms provide Ca^{2+} sensitivity to the activity-dependent increase in glucose uptake described [18,64,72,73].

Regarding the steps beyond glucose uptake two scenarios may be considered. Ca^{2+} regulation of glycolysis and mitochondrial dehydrogenases may occur independently to boost the activity of TCA activity and pyruvate use when Ca^{2+} enters in the matrix by MCU, or glycolysis and OXPHOS may be Ca^{2+}-modulated by the same mechanism, Aralar/MAS, as indicated recently [18]. Indeed, NMDA induced a rapid Ca^{2+}-dependent increase in cytosolic pyruvate levels which was blunted in AGC1/Aralar-KO neurons, and a similar increase in glucose utilization via glycolysis also abolished in the absence of AGC1/Aralar [18]. The origin of cytosolic pyruvate was glycolysis rather than uptake from the external medium. As Aralar/MAS was also required for NMDA-stimulation of respiration, the results showed that, through redox shuttling, Aralar/MAS diverts pyruvate away from lactate and into OxPhos in a Ca^{2+} regulated way. By providing NAD^+ for glycolysis, also in a Ca^{2+} dependent way, it stimulates glycolysis. Therefore, Aralar/MAS may provide a potential feed-forward mechanism linking early Ca^{2+} signals arising from synaptic activity to both glycolysis and OXPHOS, as has been suggested [71].

CRediT authorship contribution statement

Araceli del Arco: Conceptualization, Writing – review & editing. **Luis González-Moreno:** Writing – original draft. **Irene Pérez-Liébana:** Writing – original draft. **Inés Juaristi:** Writing – original draft. **Paloma González-Sánchez:** Writing – original draft. **Laura Contreras:** Resources. **Beatriz Pardo:** Resources. **Jorgina Satrústegui:** Conceptualization, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial

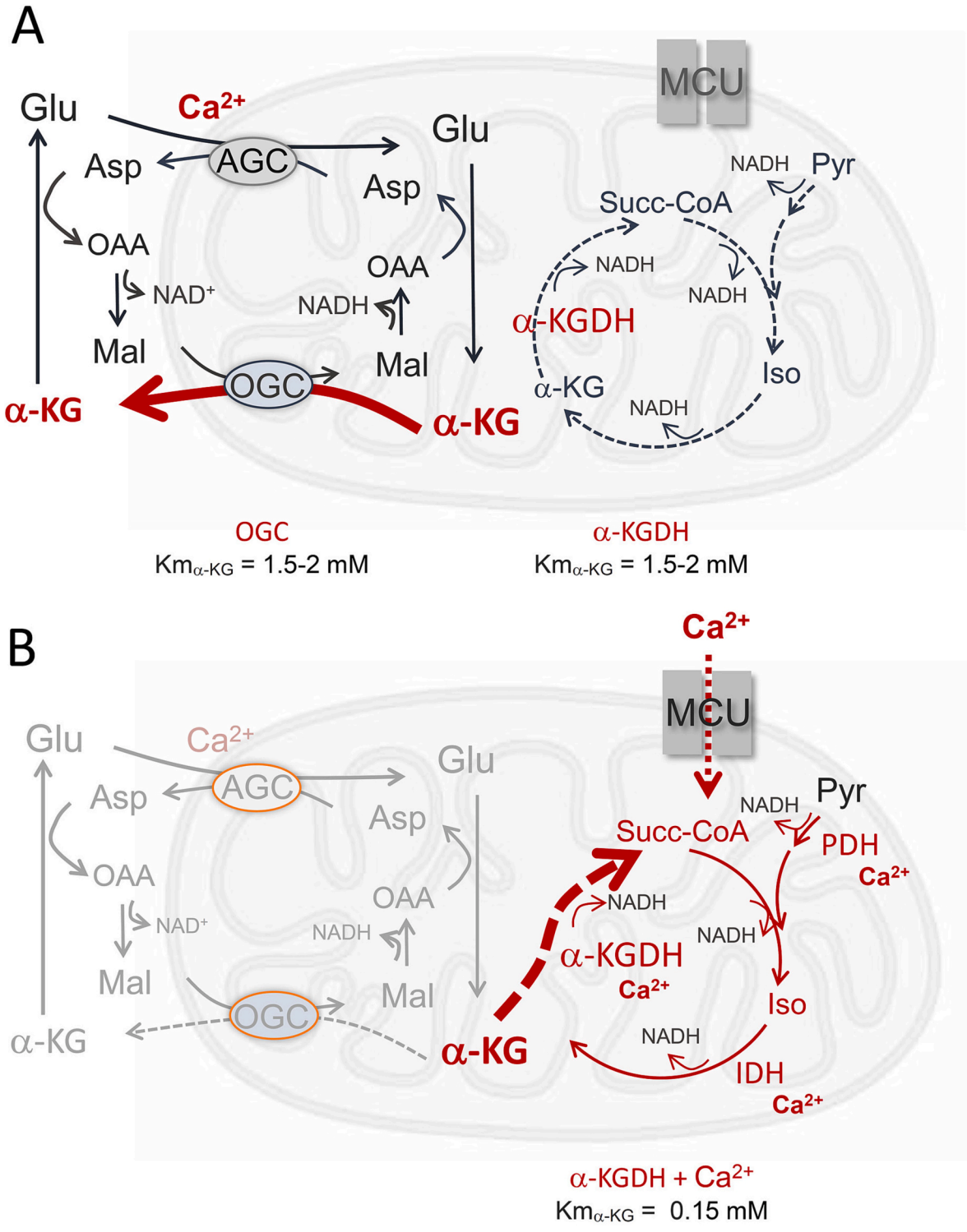

Fig. 1. Competition for mitochondrial α-ketoglutarate between MAS and TCA pathways.
Schematics of the main components and reactions of the malate/aspartate shuttle and Ca^{2+}-dependent steps of the TCA cycle. The preferential direction of mitochondrial α-KG flow in the presence or absence of Ca^{2} are shown; along MAS under basal conditions (A) and along α-KGDH (B) when Ca^{2+} levels increase after its Ca^{2+} uptake by MCU, resulting in the activation of α-KGDH with a decrease in the apparent Km for α-KG. The apparent Km for α-KG of OGC and α-KGDH under basal condition (A) and Ca^{2+}-activated conditions (B) taken from [69] are indicated. OGC, oxoglutarate-malate carrier; α-KGDH, alfa-ketoglutarate dehydrogenase.

interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgments

This work has been funded by grants from the Spanish Ministry of Science, Innovation and Universities SAF2017-82560-R and PID2020-114499RB-I00 (to AdelA and BP), and by an institutional grant from the Fundación Ramón Areces to the Centro de Biología Molecular Severo Ochoa (CBMSO).

References

[1] R.M. Denton, Regulation of mitochondrial dehydrogenases by calcium ions, Biochim. Biophys. Acta 1787 (2009) 1309–1316.
[2] B. Glancy, R.S. Balaban, Role of mitochondrial Ca2+ in the regulation of cellular energetics, Biochemistry 51 (2012) 2959–2973.
[3] J.G. McCormack, R.M. Denton, Mitochondrial Ca2+ transport and the role of intramitochondrial Ca2+ in the regulation of energy metabolism, Dev. Neurosci. 15 (1993) 165–173.
[4] C. Cárdenas, R.A. Miller, I. Smith, T. Bui, J. Molgó, M. Müller, H. Vais, K. H. Cheung, J. Yang, I. Parker, C.B. Thompson, M.J. Birnbaum, K.R. Hallows, J. K. Foskett, Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca2+ transfer to mitochondria, Cell 142 (2010) 270–283.
[5] D. Attwell, S.B. Laughlin, An energy budget for signaling in the grey matter of the brain, J. Cereb. Blood Flow Metab. 21 (2001) 1133–1145.
[6] J. Satrústegui, B. Pardo, A. Del Arco, Mitochondrial transporters as novel targets for intracellular calcium signaling, Physiol. Rev. 87 (2007) 29–67.
[7] A. del Arco, J. Satrústegui, Molecular cloning of aralar, a new member of the mitochondrial carrier superfamily that binds calcium and is present in human muscle and brain, J. Biol. Chem. 273 (1998) 23327–23334.
[8] A. del Arco, J. Satrústegui, Identification of a novel human subfamily of mitochondrial carriers with calcium-binding domains, J. Biol. Chem. 279 (2004) 24701–24713.
[9] A. del Arco, L. Contreras, B. Pardo, J. Satrustegui, Calcium regulation of mitochondrial carriers, Biochim. Biophys. Acta 1863 (2016) 2413–2421.
[10] E. Carafoli, J. Krebs, Why Calcium? How calcium became the best communicator, J. Biol. Chem. 291 (2023) 20849–20857.
[11] L. Contreras, J. Satrústegui, Calcium signaling in brain mitochondria: interplay of malate aspartate NADH shuttle and calcium uniporter/mitochondrial dehydrogenase pathways, J. Biol. Chem. 284 (2009) 7091–7099.
[12] P. Mármol, B. Pardo, A. Wiederkehr, A. Del Arco, C.B. Wollheim, J. Satrústegui, Requirement for aralar and its Ca2+-binding sites in Ca2+ signal transduction in mitochondria from INS-1 clonal beta-cells, J. Biol. Chem. 284 (2009) 515–524.
[13] L. Palmieri, B. Pardo, F.M. Lasorsa, A. del Arco, K. Kobayashi, M. Iijima, M. J. Runswick, J.E. Walker, T. Saheki, J. Satrústegui, F. Palmieri, Citrin and aralar1 are Ca(2+)-stimulated aspartate/glutamate transporters in mitochondria, EMBO J. 20 (2001) 5060–5069.
[14] B. Pardo, L. Contreras, A. Serrano, M. Ramos, K. Kobayashi, M. Iijima, T. Saheki, J. Satrústegui, Essential role of aralar in the transduction of small Ca2+ signals to neuronal mitochondria, J. Biol. Chem. 281 (2006) 1039–1047.
[15] C. Cardenas, A. Lovy, E. Silva-Pavez, F. Urra, C. Mizzoni, U. Ahumada-Castro, G. Bustos, F. Jaña, P. Cruz, P. Farias, E. Mendoza, H. Huerta, P. Murgas, M. Hunter, M. Rios, O. Cerda, I. Georgakoudi, A. Zakarian, J. Molgó, J.K. Foskett, Cancer cells with defective oxidative phosphorylation require endoplasmic reticulum-to-mitochondria Ca2+ transfer for survival, Sci. Signal. 13 (2020), eaay1212.
[16] R. Filadi, N.S. Leal, B. Schreiner, A. Rossi, G. Dentoni, C.M. Pinho, B. Wiehager, D. Cieri, T. Calì, P. Pizzo, M. Ankarcrona, TOM70 sustains cell bioenergetics by promoting IP3R3-mediated ER to mitochondria Ca2+ transfer, Curr. Biol. 28 (2018) 369–382.
[17] Z. Koshenov, F.E. Oflaz, M. Hirtl, B. Gottschalk, R. Rost, R. Malli, W.F. Graier, Citrin mediated metabolic rewiring in response to altered basal subcellular Ca2+ homeostasis, Commun. Biol. 5 (2022) 76.
[18] I. Pérez-Liébana, I. Juaristi, P. González-Sánchez, L. González-Moreno, E. Rial, M. Podunavac, A. Zakarian, J. Molgó, A. Vallejo-Illarramendi, L. Mosqueira-Martín, A. Lopez de Munain, B. Pardo, J. Satrústegui, A. Del Arco, A Ca2+-dependent mechanism boosting glycolysis and OXPHOS by activating aralar-malate-aspartate shuttle, upon neuronal stimulation, J. Neurosci. 42 (2022) 3879–3895.
[19] A.R. Díaz-Vegas, A. Cordova, D. Valladares, P. Llanos, C. Hidalgo, G. Gherardi, D. De Stefani, C. Mammucari, R. Rizzuto, A. Contreras-Ferrat, E. Jaimovich, Mitochondrial calcium increase induced by RyR1 and IP3R channel activation after membrane depolarization regulates skeletal muscle metabolism, Front. Physiol. 9 (2018) 791.
[20] D. Tomar, F. Jaña, Z. Dong, W.J. Quinn 3rd, P. Jadiya, S.L. Breves, C.C. Daw, S. Srikantan, S. Shanmughapriya, N. Nemani, E. Carvalho, A. Tripathi, A.M. Worth, X. Zhang, R. Razmpour, A. Seelam, S. Rhode, A.V. Mehta, M. Murray, D. Slade, S. H. Ramirez, P. Mishra, G.S. Gerhard, J. Caplan, L. Norton, K. Sharma, S. Rajan, D. Balciunas, D.S. Wijesinghe, R.S. Ahima, J.A. Baur, M. Madesh, Blockade of MCU-mediated Ca2+ uptake perturbs lipid metabolism via PP4-dependent AMPK dephosphorylation, Cell Rep. 26 (2019) 3709–3725.
[21] E. Zampese, D.L. Wokosin, P. Gonzalez-Rodriguez, J.N. Guzman, T. Tkatch, J. Kondapalli, W.C. Surmeier, K.B. D'Alessandro, D. De Stefani, R. Rizzuto, M. Iino, J.D. Molkentin, N.S. Chandel, P.T. Schumacker, D.J. Surmeier, Ca2+ channels couple spiking to mitochondrial metabolism in substantia nigra dopaminergic neurons, Sci. Adv. 8 (2022) eabp8701.
[22] A. Bartok, D. Weaver, T. Golenár, Z. Nichtova, M. Katona, S. Bánsághi, K. J. Alzayady, V.K. Thomas, H. Ando, K. Mikoshiba, S.K. Joseph, D.I. Yule, G. Csordás, G. Hajnóczky, IP3 receptor isoforms differently regulate ER-mitochondrial contacts and local calcium transfer, Nat. Commun. 10 (2019) 3726.
[23] M.P. Young, Z.T. Schug, D.M. Booth, D.I. Yule, K. Mikoshiba, G. Hajnóczky, S. K. Joseph, Metabolic adaptation to the chronic loss of Ca2+ signaling induced by KO of IP3 receptors or the mitochondrial Ca2+ uniporter, J. Biol. Chem. 298 (2022), 101436.
[24] E. Jaimovich, C. Mattei, J.L. Liberona, C. Cardenas, M. Estrada, J. Barbier, C. Debitus, D. Laurent, J. Molgó, Xestospongin B, a competitive inhibitor of IP3-mediated Ca2+ signalling in cultured rat myotubes, isolated myonuclei, and neuroblastoma (NG108-15) cells, FEBS Lett. 579 (2005) 2051–2057.
[25] P. Cruz, U. Ahumada-Castro, G. Bustos, J. Molgó, D. Sauma, A. Lovy, C. Cárdenas, Inhibition of InsP3R with xestospongin B reduces mitochondrial respiration and induces selective cell death in T cell acute lymphoblastic leukemia cells, Int. J. Mol. Sci. 22 (2021) 651.
[26] N. Galeotti, A. Quattrone, E. Vivoli, M. Norcini, A. Bartolini, C. Ghelardini, Different involvement of type 1, 2, and 3 ryanodine receptors in memory processes, Learn. Mem. 15 (2008) 315–323.
[27] K. Mallilankaraman, C. Cárdenas, P.J. Doonan, H.C. Chandramoorthy, K.M. Irrinki, T. Golenár, G. Csordás, P. Madireddi, J. Yang, M. Müller, R. Miller, J.E. Kolesar, J. Molgó, B. Kaufman, G. Hajnóczky, J.K. Foskett, M. Madesh, MCUR1 is an essential component of mitochondrial Ca2+ uptake that regulates cellular metabolism, Nat. Cell Biol. 14 (2012) 1336–1343.
[28] P.A. Egorova, I.B. Bezprozvanny, Inositol 1,4,5-trisphosphate receptors and neurodegenerative disorders, FEBS J. 285 (2018) 3547–3565.
[29] R. Jakob, G. Beutner, V.K. Sharma, Y. Duan, R.A. Gross, S. Hurst, B.S. Jhun, J. O-Uchi, S.S. Sheu, Molecular and functional identification of a mitochondrial ryanodine receptor in neurons, Neurosci Lett. 575 (2014) 7–12.
[30] G. Hajnóczky, G. Csordás, M. Yi, Old players in a new role: mitochondria-associated membranes, VDAC, and ryanodine receptors as contributors to calcium signal propagation from endoplasmic reticulum to the mitochondria, Cell Calcium 32 (2002) 363–377.
[31] K. Völgyi, K. Badics, F.J. Sialana, P. Gulyássy, E.B. Udvari, V. Kis, L. Drahos, G. Lubec, K.A. Kékesi, G. Juhász, Early presymptomatic changes in the proteome of mitochondria-associated membrane in the APP/PS1 mouse model of Alzheimer's disease, Mol. Neurobiol. 55 (2018) 7839–7857.
[32] M. Suman, J.A. Sharpe, R.B. Bentham, V.N. Kotiadis, M. Menegollo, V. Pignataro, J. Molgó, F. Muntoni, M.R. Duchen, E. Pegoraro, G. Szabadkai, Inositol trisphosphate receptor-mediated Ca2+ signalling stimulates mitochondrial function and gene expression in core myopathy patients, Hum. Mol. Genet. 27 (2018) 2367–2382.
[33] X. Pan, J. Liu, T. Nguyen, C. Liu, J. Sun, Y. Teng, M.M. Fergusson, I.I. Rovira, M. Allen, D.A. Springer, A.M. Aponte, M. Gucek, R.S. Balaban, E. Murphy, T. Finkel, The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter, Nat. Cell Biol. 15 (2013) 1464–1472.
[34] C.M. Bisbach, R.A. Hutto, D. Poria, W.M. Cleghorn, F. Abbas, F. Vinberg, V. J. Kefalov, J.B. Hurley, S.E. Brockerhoff, Mitochondrial calcium uniporter (MCU) deficiency reveals an alternate path for Ca2+ uptake in photoreceptor mitochondria, Sci. Rep. 10 (2020) 16041.
[35] C.M. Díaz-García, D.J. Meyer, N. Nathwani, M. Rahman, J.R. Martínez-François, G. Yellen, The distinct roles of calcium in rapid control of neuronal glycolysis and the tricarboxylic acid cycle, elife 10 (2021), e64821.
[36] A. Kosmach, B. Roman, J. Sun, A. Femnou, F. Zhang, C. Liu, C.A. Combs, R. S. Balaban, E. Murphy, Monitoring mitochondrial calcium and metabolism in the beating MCU-KO heart, Cell Rep. 37 (2021), 109846.
[37] M. Nichols, P.A. Elustondo, J. Warford, A. Thirumaran, E.V. Pavlov, G. S. Robertson, Global ablation of the mitochondrial calcium uniporter increases glycolysis in cortical neurons subjected to energetic stressors, J. Cereb. Blood Flow Metab. 37 (2017) 3027–3041.
[38] M. Nichols, E.V. Pavlov, G.S. Robertson, Tamoxifen-induced knockdown of the mitochondrial calcium uniporter in Thy1-expressing neurons protects mice from hypoxic/ischemic brain injury, Cell Death Dis. 9 (2018) 606.
[39] M. Szibor, Z. Gizatullina, T. Gainutdinov, T. Endres, G. Debska-Vielhaber, M. Kunz, N. Karavasili, K. Hallmann, F. Schreiber, A. Bamberger, M. Schwarzer, T. Doenst, H.J. Heinze, V. Lessmann, S. Vielhaber, W.S. Kunz, F.N. Gellerich, Cytosolic, but not matrix, calcium is essential for adjustment of mitochondrial pyruvate supply, J. Biol. Chem. 295 (2020) 4383–4397.
[40] P. Álvarez-Illera, P. García-Casas, R.I. Fonteriz, M. Montero, J. Alvarez, Mitochondrial Ca2+ dynamics in MCU Knockout C. Elegans Worms, Int. J. Mol. Sci. 21 (2020) 8622.
[41] E. Murphy, X. Pan, T. Nguyen, J. Liu, K.M. Holmström, T. Finkel, Unresolved questions from the analysis of mice lacking MCU expression, Biochem. Biophys. Res. Commun. 449 (2014) 384–385.
[42] T.P. Rasmussen, Y. Wu, M.L. Joiner, O.M. Koval, N.R. Wilson, E.D. Luczak, Q. Wang, B. Chen, Z. Gao, Z. Zhu, B.A. Wagner, J. Soto, M.L. McCormick, W. Kutschke, R.M. Weiss, L. Yu, R.L. Boudreau, E.D. Abel, F. Zhan, D.R. Spitz, G.

R. Buettner, L.S. Song, L.V. Zingman, M.E. Anderson, Inhibition of MCU forces extramitochondrial adaptations governing physiological and pathological stress responses in heart, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 9129–9134.
[43] P. Wang, C. Fernandez-Sanz, W. Wang, S.S. Sheu, Why don't mice lacking the mitochondrial Ca2+ uniporter experience an energy crisis? J. Physiol. 598 (2020) 1307–1326.
[44] H. Wu, B. Brand, M. Eckstein, S.M. Hochrein, M. Shumanska, J. Dudek, A. Nickel, C. Maack, I. Bogeski, M. Vaeth, Genetic ablation of the mitochondrial calcium uniporter (MCU) does not impair T cell-mediated immunity in vivo, Front. Pharmacol. 12 (2021), 734078.
[45] C. Bas-Orth, J. Schneider, A. Lewen, J. McQueen, K. Hasenpusch-Theil, T. Theil, G. E. Hardingham, H. Bading, O. Kann, The mitochondrial calcium uniporter is crucial for the generation of fast cortical network rhythms, J. Cereb. Blood Flow Metab. 40 (2020) 2225–2239.
[46] J.Q. Kwong, X. Lu, R.N. Correll, J.A. Schwanekamp, R.J. Vagnozzi, M.A. Sargent, A.J. York, J. Zhang, D.M. Bers, J.D. Molkentin, The mitochondrial calcium uniporter selectively matches metabolic output to acute contractile stress in the heart, Cell Rep. 12 (2015) 15–22.
[47] D. Flicker, Y. Sancak, E. Mick, O. Goldberger, V.K. Mootha, Exploring the in vivo role of the mitochondrial calcium uniporter in Brown fat bioenergetics, Cell Rep. 27 (2019) 1364–1375.e5.
[48] J.C. Liu, N.C. Syder, N.S. Ghorashi, T.B. Willingham, R.J. Parks, J. Sun, M. M. Fergusson, J. Liu, K.M. Holmström, S. Menazza, D.A. Springer, C. Liu, B. Glancy, T. Finkel, E. Murphy, EMRE is essential for mitochondrial calcium uniporter activity in a mouse model, JCI Insight. 5 (2020), e134063.
[49] R. Tufi, T.P. Gleeson, S. von Stockum, V.L. Hewitt, J.J. Lee, A. Terriente-Felix, A. Sanchez-Martinez, E. Ziviani, A.J. Whitworth, Comprehensive genetic characterization of mitochondrial Ca2+ uniporter components reveals their different physiological requirements in vivo, Cell Rep. 27 (2019) 1541–1550.
[50] E.P. Bulthuis, M.J. Adjobo-Hermans, B. de Potter, S. Hoogstraten, L.H. Wezendonk, O.A. Tutakhel, L.T. Wintjes, B. van den Heuvel, P.H. Willems, E. Kamsteeg, M. E. Rubio Gozalbo, S.C. Sallevelt, S.M. Koudijs, J. Nicolai, C.I. de Bie, J. E. Hoogendijk, W.J. Koopman, R.J. Rodenburg, SMDT1Variants Impair EMRE-mediated Mitochondrial Calcium Uptake in Patients With Muscle Involvement 2022, bioRxiv, 2022, https://doi.org/10.1101/2022.10.31.514480.
[51] G. Csordás, T. Golenár, E.L. Seifert, K.J. Kamer, Y. Sancak, F. Perocchi, C. Moffat, D. Weaver, S.F. Perez, R. Bogorad, V. Koteliansky, J. Adijanto, V.K. Mootha, G. Hajnóczky, MICU1 controls both the threshold and cooperative activation of the mitochondrial Ca2+ uniporter, Cell Metab. 17 (2013) 976–987.
[52] F. Perocchi, V.M. Gohil, H.S. Girgis, X.R. Bao, J.E. McCombs, A.E. Palmer, V. K. Mootha, MICU1 encodes a mitochondrial EF hand protein required for Ca(2+) uptake, Nature 467 (2010) 291–296.
[53] A. Dhoundiyal, V. Goeschl, S. Boehm, H. Kubista, M. Hotka, Glycerol-3-phosphate shuttle is a backup system securing metabolic flexibility in neurons, J. Neurosci. 42 (2022) 7339–7354.
[54] I. Llorente-Folch, C.B. Rueda, I. Amigo, A. del Arco, T. Saheki, B. Pardo, J. Satrústegui, Calcium-regulation of mitochondrial respiration maintains ATP homeostasis and requires ARALAR/AGC1-malate aspartate shuttle in intact cortical neurons, J. Neurosci. 33 (2013) 13957–13971.
[55] I. Llorente-Folch, C.B. Rueda, B. Pardo, G. Szabadkai, M.R. Duchen, J. Satrustegui, The regulation of neuronal mitochondrial metabolism by calcium, J. Physiol. 593 (2015) 3447–3462.
[56] I. Juaristi, M.L. García-Martín, T.B. Rodrigues, J. Satrústegui, I. Llorente-Folch, B. Pardo, ARALAR/AGC1 deficiency, a neurodevelopmental disorder with severe impairment of neuronal mitochondrial respiration, does not produce a primary increase in brain lactate, J. Neurochem. 142 (2017) 132–139.
[57] C.B. Rueda, I. Llorente-Folch, I. Amigo, L. Contreras, P. González-Sánchez, P. Martínez-Valero, I. Juaristi, B. Pardo, A. del Arco, J. Satrústegui, Ca(2+) regulation of mitochondrial function in neurons, Biochim. Biophys. Acta 1837 (2014) 1617–1624.
[58] I. Juaristi, L. Contreras, P. González-Sánchez, I. Pérez-Liébana, L. González-Moreno, B. Pardo, A. Del Arco, J. Satrústegui, The response to stimulation in neurons and astrocytes, Neurochem. Res. 44 (2019) 2385–2391.
[59] L. Contreras, P. Gomez-Puertas, M. Iijima, K. Kobayashi, T. Saheki, J. Satrústegui, Ca2+ activation kinetics of the two aspartate-glutamate mitochondrial carriers, aralar and citrin: role in the heart malate-aspartate NADH shuttle, J. Biol. Chem. 282 (2007) 7098–7106.
[60] F.N. Gellerich, Cytosolic, but not matrix, calcium is essential for adjustment of mitochondrial pyruvate supply, J. Biol. Chem. 295 (2020) 4383–4397.
[61] F.N. Gellerich, Z. Gizatullina, T. Gainutdinov, K. Muth, E. Seppet, Z. Orynbayeva, S. Vielhaber, The control of brain mitochondrial energization by cytosolic calcium: the mitochondrial gas pedal, IUBMB Life 65 (2013) 180–190.
[62] F.N. Gellerich, Z. Gizatullina, S. Trumbekaite, B. Korzeniewski, T. Gaynutdinov, E. Seppet, S. Vielhaber, H.J. Heinze, F. Striggow, Cytosolic Ca2+ regulates the energization of isolated brain mitochondria by formation of pyruvate through the malate-aspartate shuttle, Biochem. J. 443 (2012) 747–755.
[63] R. Arce-Molina, F. Cortés-Molina, P.Y. Sandoval, A. Galaz, K. Alegría, S. Schirmeier, L.F. Barros, Martín A. San, A highly responsive pyruvate sensor reveals pathway-regulatory role of the mitochondrial pyruvate carrier MPC, elife 9 (2020), e53917.
[64] G. Ashrafi, Z. Wu, R.J. Farrell, T.A. Ryan, GLUT4 mobilization supports energetic demands of active synapses, Neuron 93 (2017) 606–615.
[65] L.S. Jouaville, P. Pinton, C. Bastianutto, G.A. Rutter, R. Rizzuto, Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 13807–13812.
[66] G. Ashrafi, J. de Juan-Sanz, R.J. Farrell, T.A. Ryan, Molecular tuning of the axonal mitochondrial Ca2+ uniporter ensures metabolic flexibility of neurotransmission, Neuron 105 (2020) 678–687.
[67] I. Pérez-Liébana, M.J. Casarejos, A. Alcaide, E. Herrada-Soler, I. Llorente-Folch, L. Contreras, J. Satrústegui, B. Pardo, βOHB protective pathways in aralar-KO neurons and brain: an alternative to ketogenic diet, J. Neurosci. 40 (2020) 9293–9305.
[68] J. Satrústegui, L.K. Bak, Fluctuations in cytosolic calcium regulate the neuronal malate-aspartate NADH shuttle: implications for neuronal energy metabolism, Neurochem. Res. 40 (2015) 2425–2430.
[69] J.G. McCormack, R.M. Denton, The effects of calcium ions and adenine nucleotides on the activity of pig heart 2-oxoglutarate dehydrogenase complex, Biochem. J. 180 (1979) 533–544.
[70] D. De Stefani, R. Rizzuto, T. Pozzan, Enjoy the trip: calcium in mitochondria Back and forth, Annu. Rev. Biochem. 85 (2016) 161–192.
[71] G. Ashrafi, T.A. Ryan, Glucose metabolism in nerve terminals, Curr. Opin. Neurobiol. 45 (2017) 156–161.
[72] N.M. Connolly, H. Düssmann, U. Anilkumar, H.J. Huber, J.H. Prehn, Single-cell imaging of bioenergetic responses to neuronal excitotoxicity and oxygen and glucose deprivation, J. Neurosci. 34 (2014) 10192–10205.
[73] J.M. Ferreira, A.L. Burnett, G.A. Rameau, Activity-dependent regulation of surface glucose transporter-3, J. Neurosci. 31 (2011) 1991–1999.
[74] J. Kawashima, T. Alquier, Y. Tsuji, O.D. Peroni, B.B. Kahn, Ca2+/calmodulin-dependent protein kinase kinase is not involved in hypothalamic AMP-activated protein kinase activation by neuroglucopenia, PLoS One 7 (2014), e36335.