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Minireview

Mitochondria and L-lactate metabolism

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Abstract Although mitochondria have been the object of intensive study over many decades, some aspects of their metabolism remain to be fully elucidated, including the L-lactate metabolism.

We review here the novel insights arisen from investigations on L-lactate metabolism in mammalian, plant and yeast mitochondria. The presence of L-lactate dehydrogenases inside mitochondria, where L-lactate enters in a carrier-mediated fashion, suggests that mitochondria play an important role in L-lactate metabolism. Functional studies have demonstrated the occurrence of several L-lactate carriers. Moreover, immunological investigations have proven the existence of monocarboxylate translocator isoforms in mitochondria.
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1. Mitochondrial metabolism of L-lactate in mammals

A fundamental change in the overall view of the role of L-lactate (L-LAC) in metabolism occurred when it was shown that O_2 limitation is not a requirement for net formation, and that L-LAC is an important intermediary in glucose metabolism, a mobile fuel for aerobic metabolism, perhaps a mediator of redox state among various compartments both within and between cells [1]. Moreover, very recently, L-LAC was proposed to work as a signal in gene expression [2].

Recently, L-LAC metabolism has been the subject of several reviews [1,3–5 and refs. therein] and consequently we will limit the scope of this review to the role of mitochondria in L-LAC metabolism. Indeed, even though mitochondrial L-LAC metabolism was originally reported as early as 1959 [6], whether and how mitochondria participate to L-LAC metabolism has mostly been investigated only in the last two decades (Table 1). Contrarily, evidence of the existence of mitochondrial L-LAC carriers, as functionally investigated, was shown only in 2002 [7]. Our present position, based on our own work and that of others reviewed here is that the evidence for mitochondrial metabolism of L-LAC, due to the existence of both L-LAC carrier-mediated transport processes and of the mitochondrial L-lactate dehydrogenase (mL-LDH) is now compelling.

1.1. Spermatozoa

The first evidence in favour of the presence of a putative mL-LDH was from Clausen who found that about 40% of total activity in sperm cells was present in a particulate fraction showing high succinate dehydrogenase activity [8].

The existence of the mL-LDH which can reduce pyridine nucleotide in the mitochondrial matrix was demonstrated in hypotonically treated rabbit epididymal spermatozoa by means of oxygen uptake and fluorimetric studies [9]. It was also shown that the mL-LDH functions actively in these cells. In sperm cells the cytosolic L-lactate dehydrogenase (cL-LDH) and mL-LDH appear to be the same isoenzyme unique to those cells: L-LDH-X. The dual localization of the enzyme enables mammalian spermatozoa to exchange cytosolic and mitochondrial reducing equivalents by means of a L-LAC/pyruvate (PYR) shuttle, as suggested by Blanco et al. [10] and reconstituted later [11].

1.2. Liver

Since liver possesses the enzymatic machinery for gluconeogenesis (GNG) and since L-LAC is a major substrate for GNG, liver play a major role in L-LAC metabolism. Indeed, metabolism of L-LAC has traditionally been considered solely as a function of the cL-LDH in spite of the fact that the L-LAC oxidation by mitochondria and the existence of an mL-LDH in the inner compartments of isolated rat liver mitochondria (RLM) has been widely reported [12–15]. In a recent detailed investigation de Bari et al. [16] showed that externally added L-LAC can be oxidized in the matrix by an mL-LDH, with reduction of intramitochondrial $NAD(P)^+$ and generation of a mitochondrial electrochemical proton gradient. Mitochondrial L-LAC transport was investigated and the rate of L-LAC metabolism in vitro was found to be limited by the rate of L-LAC transport into the mitochondria. Three separate L-LAC translocators, distinct from those which translocate

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Abbreviations: cL-LDH, cytosolic L-lactate dehydrogenase; COX, cytochrome *c* oxidase; GNG, gluconeogenesis; L-LAC, L-lactate; mL-LDH, mitochondrial L-lactate dehydrogenase; MCT, monocarboxylate transporter; MCT1, monocarboxylate transporter isoform 1; MCT2, monocarboxylate transporter isoform 2; MPC, mitochondrial pyruvate carrier; OAA, oxaloacetate; PYR, pyruvate; RHM, rat heart mitochondria; RLM, rat liver mitochondria

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Table 1
The history of the L-lactate-mitochondria affair

Authors	Year	Source	Experimental approach	Main conclusions	Refs
Sacktor	1959	RBM	Oxygen uptake by polarography	RBM can oxidize L-LAC	[6]
Clausen	1969	Human sperm cells and rat tests	L-LDH and SDH spectrophotometric assay	L-LDH X isoenzyme is associated with SDH	[8]
Baba and Sharma	1971	RHM and RSMM	Histochemical and EM techniques	L-LDH is associated with the inner membrane and located in the mitochondrial matrix	[24]
Skilleter and Kun	1972	RLM	RLM fractionation with digitonin	NAD^+-dependent L-LDH is found in the intermembrane space fraction	[12]
Blanco et al.	1976	Mouse testes	L-LDH spectrophotometric assay	L-LDH X is located in the heavy fraction of mitochondria	[10]
Storey and Kayne	1977	HTRES	Oxygen uptake by polarography and fluorimetric measurements	Existence of an L- LDH in the mitochondrial matrix	[9]
Kline et al.	1986	RLM	Oxygen uptake by polarography	L-LDH is located in mitochondria	[13]
Brandt et al.	1987	RHM, RLM, RKM and RLYM	RLM fractionation with digitonin and treatment with subtilisin	L-LDH is located in the inner mitochondrial compartments	[14]
Szczesna-Kaczmarek	1990	RSMM	Oxygen uptake by polarography	L-LAC is oxidized by RSMM in a manner sensitive to oxamate and respiratory chain inhibitors	[32]
Popinigis et al.	1991	HSMM	Oxygen uptake by polarography	No evidence of m-L-LDH or L-LAC oxidation by mitochondria	[39]
Laughlin et al.	1993	Dog heart	Isotopic measurements	L-LAC metabolism takes place in mitochondria	[25]
Gallina et al.	1994	Mouse, rat and rabbit STM	Spectrophotometric assay	In vitro reconstruction of L-LAC/PYR shuttle	[11]
Izumi et al.	1997	Rat hippocampal slices	Histological measurements	L-LAC is an adequate energy substrate for sustaining brain function	[48]
Brooks et al.	1999	RLM, RHM and RSMM	Polarographic measurements and electrophoretic analysis	An intramitochondrial L-LDH pool facilitates L-LAC oxidation	[15]
Nakae et al.	1999	Muscle fibers of mdx gastrocnemius	Confocal microscopy and immunofluorescence analysis	L-LDH colocalizes with SDH	[35]
Dubouchaud et al.	2000	HSM	Immunoblotting analysis	Human mitochondrial preparations contain L-LDH	[36]
McClelland and Brooks	2002	RHG, RSRG, RSWG	Immunoblotting analysis	Rat mitochondrial preparations contain L-LDH	[37]
Valenti et al.	2002	RHM	Spectrophotometric assay	In vitro reconstruction of L-LAC/PYR shuttle	[7]
de Bari et al.	2004	RLM	Oxygen uptake by polarography, fluorimetric measurements of $\Delta\psi$ generation and L-LAC uptake by RLM, potentiometric measurements of H^+ uptake	L-LAC is taken up by RLM in a carrier-mediated manner and oxidized inside RLM, providing OAA outside RLM with partial GNG reconstituted	[16]
Sahlin et al.	2002	RSMM	Oxygen uptake by polarography and electrophoretic measurements	No evidence of an intracellular L-LAC shuttle	[40]
Rasmussen et al.	2002	HSM and MSM	Oxygen uptake by polarography and electrophoretic measurements	L-LDH is not a mitochondrial enzyme	[41]
Ponsot et al.	2005	HSF, MSF	In situ study of mitochondrial oxygen uptake	No sign of direct mitochondrial L-LAC oxidation	[42]

Table 1 (*continued*)

Authors	Year	Source	Experimental approach	Main conclusions	Refs
Hashimoto et al.	2006	Skeletal L6 muscle cells	Immunoblotting and immunoprecipitation	Evidence of a mitochondrial L-LAC oxidation complex	[30]
Yoshida et al.	2007	Red and white RSMM	Oxygen uptake by polarography	Negligible L-LAC oxidation by mitochondria	[43]
Schurr and Payne	2007	Rat hippocampal slice	Electrophysiological measurements	L-LAC, not PYR, is neuronal aerobic glycolysis end product	[49]
Atlante et al.	2007	RCGM	Oxygen uptake by polarography spectrophotometric measurements and immunoblotting analysis	Transport and metabolism of L-LAC occurs in RCGM	[52]
Hashimoto et al.	2007	L6 cells	Screening of genome wide responses	L-LAC as a signal for gene expression	[2]
Hashimoto et al.	2008	Rat cortical, hippocampal and thalamic neurons	Immunohistochemical analyses immunoblotting and immunoprecipitation	MCT1, MCT2, and LDH colocalize with COX	[53]
Lemire et al.	2008	Human astrocytoma cell line	Oxygen uptake, immunoblotting fluorescence microscopy	mL-LDH is involved in oxidative-energy metabolism in human astrocytoma cells	[54]

Main abbreviations: EM, electron microscopy; HSM, human skeletal muscle; HSF, heart skinned fibers; HSMM, human skeletal muscle mitochondria; HTRES, hypotonically treated rabbit epididymal spermatozoa; MSF, muscle skinned fibers; MSM, mouse skeletal muscle; RHG, rat heart gastrocnemius; RHM, rat heart mitochondria; RKM, rat kidney mitochondria; RLM, rat liver mitochondria; RLYM, rat lymphocyte mitochondria; RCGM, rat cerebellar granule mitochondria; RSMM, rat skeletal muscle mitochondria; RSRG, rat soleus red gastrocnemius; RSWG, rat soleus white gastrocnemius; SDH, succinate dehydrogenase; STM, sperm-type mitochondria.

pyruvate (PYR carrier, since 2003 named mitochondrial pyruvate carrier (MPC) [17]) or D-lactate [18], were functionally investigated: the L-LAC/H^+ symporter, which loads mitochondria with L-LAC, and the L-LAC/oxaloacetate (L-LAC/OAA) and the LAC/PYR antiporters, which mediate the efflux from mitochondria of either OAA or PYR, respectively, newly synthesized as a result of mitochondrial metabolism of L-LAC. Clearly, the ability of L-LAC to export OAA out of the mitochondria and thus to trigger GNG, provides a previously unrecognised route to hepatic GNG and this route was partially reconstructed in vitro (see [16] and Scheme 1). On the other hand the L-LAC/PYR shuttle (see also [19]) was reconstructed in vitro from the combined actions of the c- and mL-LDH and of the L-LAC/PYR antiporter.

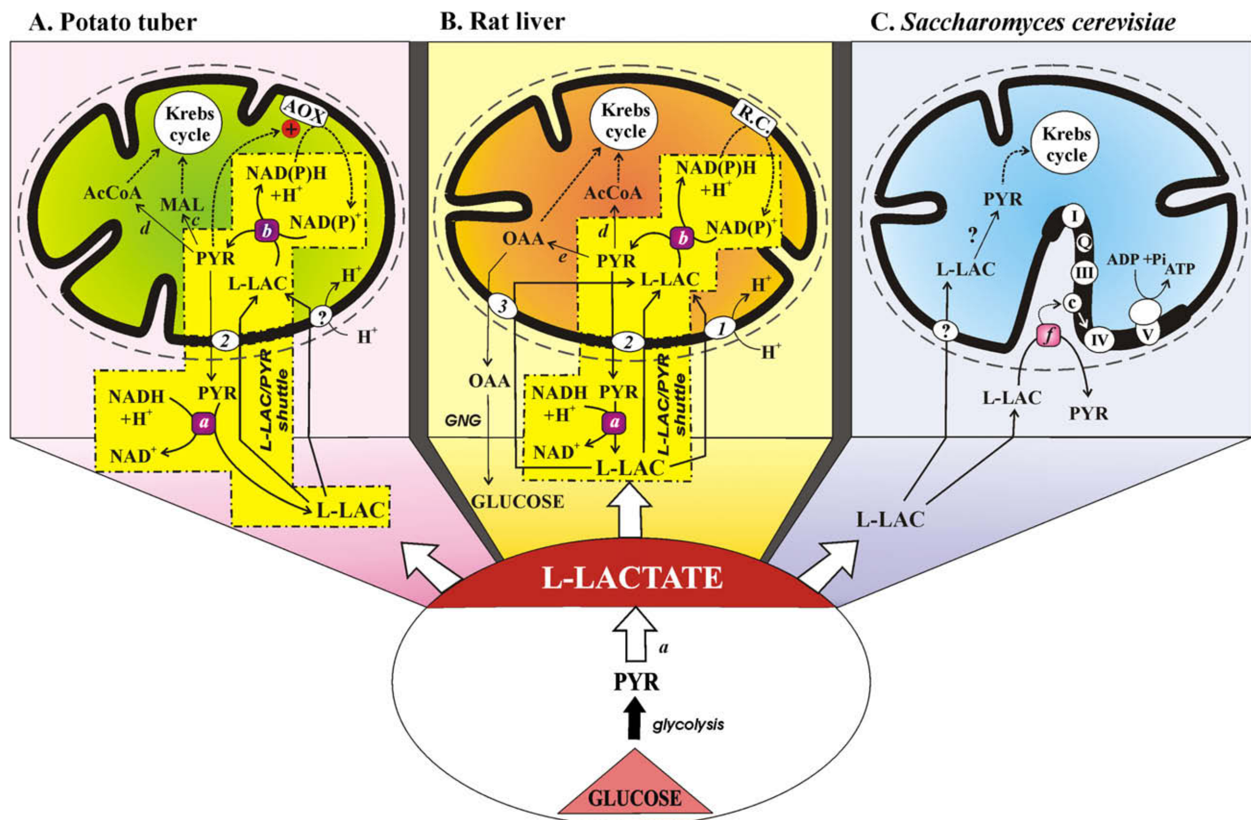
Notice that at low L-LAC concentration, but not at 10 mM L-LAC, the rate of GNG is determined by PYR transport; this argues for both excess capacity and a significant alternative pathway, perhaps the L-LAC/mitochondria pathway [20].

1.3. Heart

The arterial L-LAC concentration is low in mammals during sustained exercise because its production is balanced not only by its well known use in GNG but, more importantly for the present discussion, by mitochondrial oxidation [21]. Heart is an active L-LAC consumer: healthy myocardium simultaneously consumes and produces L-LAC even under resting conditions and it takes up L-LAC in proportion to the rate of L-LAC delivery to the myocardium both at rest and during exercise [22]. Consistently, when the arterial L-LAC concentration increases it becomes the preferred fuel for the heart with essentially all of the L-LAC taken up during exercise immediately oxidized to CO_2 in the myocardium [22,23]. The traditional view has been that L-LAC oxidation occurs via the cL-LDH reaction and that the PYR produced is oxidized in mitochondria. However, about 20 years after the first report of the existence of mL-LDH in heart [24], Laughlin [25] showed that in working canine hearts, in distinction with [^{13}C] PYR, infusion of [^{13}C]L-LAC resulted in labelling of the mitochondrial substrate 2-oxoglutarate but not of cytosolic PYR or alanine, clearly showing the occurrence of intramitochondrial L-LAC metabolism. Confirmatory proof that rat heart mitochondria (RHM) can metabolize L-LAC was given by Valenti et al. [7] who successfully reconstructed the L-LAC/PYR shuttle which occurs in a manner not inhibited by low concentrations of α-cyano-4-hydroxycinnamate, this showing the occurrence also in RHM of carrier/s separate from MPC [17].

1.4. Skeletal muscle

Until recently L-LAC accumulation in skeletal muscle was mostly considered as a consequence of anaerobic metabolism, which occurs when the need for tissues to generate energy exceeds their capacity to oxidize the PYR produced by glycolysis. In fact, skeletal muscle can take up L-LAC from the blood or produce it from as much as 50% of the metabolized glucose, even under fully aerobic conditions [26–28]. In particular, a "shuttling" of oxidisable substrate in the form of L-LAC has been proposed, from areas of high glycogenolytic rate to areas of high cellular respiration both at rest and during exercise [27]. The occurrence of an L-LAC oxidation by muscle mitochondrial reticulum [29] has been proposed by many authors (see Table 1). On the basis of the existence of a mL-LDH [13–16], Brooks and colleagues [15] proposed the intracellular L-lactate shuttle (different from the L-LAC/PYR shuttle) hypothesis which posits that L-LAC produced as the result of glycolysis and glycogenolysis in the cytosol is balanced by oxidation in mitochondria of the same cell. As an extension to this idea it was suggested that glycolytically derived PYR



Scheme 1. The mitochondrial metabolism of L-lactate. (A) The mitochondrial metabolism of L-lactate in potato tuber. The sequence of events involved in mitochondrial metabolism of L-lactate is envisaged as: uptake into mitochondria of L-LAC, synthesized in the cytosol by anaerobic glycolysis, perhaps via the L-LAC/H^+ symporter; oxidation of the L-LAC to PYR by the mL-LDH located in the inner mitochondrial compartment; activation of alternative oxidase (AOX) by the newly synthesized PYR, oxidation of the intramitochondrial NAD(P)H via AOX with efflux of PYR via a putative L-LAC/PYR antiporter and the oxidation of cytosolic NADH in a non-energy-competent L-LAC/PYR shuttle. PYR conversion could also occur to AcetylCoA and malate via pyruvate dehydrogenase and malic enzyme, respectively. (B) The mitochondrial metabolism of L-lactate in liver. Externally added L-LAC can enter RLM and cause the efflux in the extramitochondrial phase of PYR and OAA newly synthesised in the mitochondrial matrix via mL-LDH and pyruvate carboxylase. The metabolite efflux occurs by virtue of the occurrence of three carriers for L-LAC transport in mitochondria: the L-LAC/H^+ symporter and the L-LAC/PYR and L-LAC/OAA antiporters. The LAC/PYR antiporter accounts for the LAC/PYR shuttle which transfers reducing equivalents from the cytoplasm to mitochondrial respiratory chain. The L-LAC/OAA antiporter accounts for a novel GNG. OAA and PYR (via the pyruvate dehydrogenase) could also fill up the Krebs cycle intermediate pool. (C) The mitochondrial metabolism of L-lactate in *Saccharomyces cerevisiae*. Externally added L-LAC can enter mitochondria via a putative L-LAC/H^+ symporter. In mitochondria an NAD- dependent mL-LDH exists. Moreover, in the intermembrane space L-LAC is oxidized to PYR with reduction of cytochrome *c* by a flavin mitochondrial L(+)-lactate: cytochrome *c* oxidoreductase in a energy competent manner. *Abbreviations*: AcCoA, acetyl-CoA; AOX, alternative oxidase; GNG, gluconeogenesis; L-LAC, L-lactate; MAL, malate; OAA, oxaloacetate; PYR, pyruvate; R.C., respiratory chain; ?, transport and oxidation processes the existence of which has not yet been confirmed. Enzymes: *a*, cytosolic L-LDH; *b*, mitochondrial L-LDH; *c*, malic enzyme; *d*, pyruvate dehydrogenase; *e*, pyruvate carboxylase; *f*, L-lactate: cytochrome *c* oxidoreductase (*Cyb2p*). Mitochondrial carriers: 1, L-LAC/H^+ symporter; 2, L-LAC/PYR antiporter; 3, L-LAC/OAA antiporter.

is preferentially metabolized to L-LAC in the cytosol rather than to acetyl-CoA after its carrier-mediated uptake by mitochondria [15,30–34].

In addition to a variety of enzymatic assays of mL-LDH, in a series of papers from Brooks' group the existence of the mL-LDH has been shown by using confocal microscopy and immunofluorescence analyses [15,30]; moreover, succinate dehydrogenase and mL-LDH were reported to colocalize in mouse muscle [35]. Additionally, mitochondrial preparations from human [36] and rat [37] both appear to contain mL-LDH, as demonstrated by Western blotting analysis. That mL-LDH is anchored on the outer side of the inner mitochondrial membrane in mitochondria from skeletal muscle cells and the existence of a "mitochondrial lactate oxidation complex" was also reported [30,38].

In contrast, other groups failed to show either mL-LDH or L-LAC oxidation by mitochondria and it was claimed that skeletal muscle mitochondria do not contain significant amounts of L-LDH activity [39–43] (Table 1). A vigorous debate ensued [43–46] but the space limitation of this minireview does not allow us to enter into that debate here. However, we recently isolated mitochondria from rabbit gastrocnemius in the absence of protease and found that externally added L-LAC caused an increase in fluorescence of intramitochondrial NAD(P)H. In parallel, the occurrence of an mL-LDH was shown by immunological analysis and enzyme assay in solubilized mitochondria. Surprisingly enough, these results could not be obtained when proteinase K was used in the isolation procedure (unpublished data). Thus, in agreement with [47], we maintain that skeletal muscle mitochondria contain

their own mL-LDH. Both fluorescence experiments and the failure to assay mL-LDH in intact mitochondria strongly suggest that mL-LDH is located in the inner compartments also in skeletal muscle mitochondria, thus needing L-LAC transport. Although to date L-LAC transport in skeletal muscle has not been investigated in isolated coupled organelles, the occurrence of monocarboxylate transporter (MCT) isoforms was shown in mitochondria from different muscles [47].

1.5. Brain

Evidence has accumulated over the last two decades indicating that L-LAC is an important cerebral oxidative-energy substrate (for refs. see [4,48,49]): brain can take up L-LAC from blood, particularly during intense exercise, as well as in the initial minutes of recovery [50]. Moreover, an "astrocyte–neuron L-LAC shuttle" has been proposed, in which astrocytes take up glucose from blood, convert it into L-LAC via glycolysis and then export L-LAC into the extracellular phase via the isoform 1 of monocarboxylate transporter (MCT1). In turn neurons take up extracellular L-LAC via the isoform 2 of monocarboxylate transporter (MCT2) and use it as a fuel for mitochondrial respiration [51].

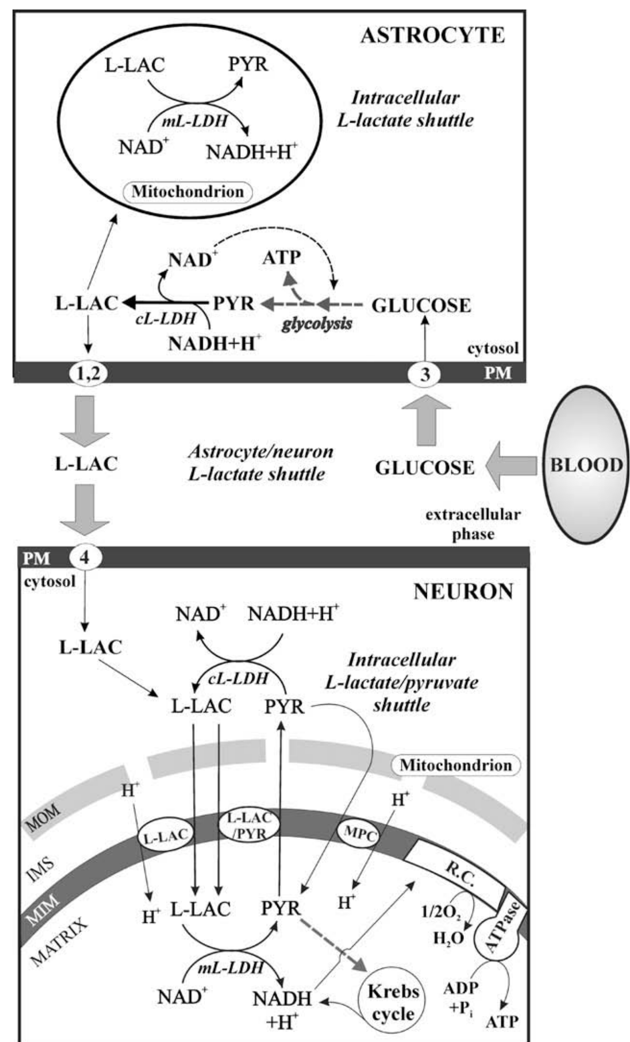
Recently it was hypothesized that, in the brain, L-LAC is the principal product of glycolysis, whether or not oxygen is present [34].

These observations again raise the question of mitochondrial involvement in oxidation of L-LAC by brain and evidence presented in 1959 by Sacktor et al. [6] suggests that this does indeed occur. After nearly 50 years, we have confirmed that neurons (in particular cerebellar granule cells) can take up and metabolize externally added L-LAC. L-LAC metabolism can occur also in mitochondria from these cells: the existence of an mL-LDH located in the inner mitochondrial compartment was shown by both immunological and enzymatic analysis. Consistently, externally added L-LAC enters mitochondria, perhaps via an L-LAC/H^+ symporter, and is oxidized in a manner stimulated by ADP [52]. As in [7,11,16], an L-LAC/PYR shuttle operates in neurons (Scheme 2). Whether these translocators are those found in mitochondrial reticulum by Hashimoto et al., who showed the presence of mL-LDH and MCT1 and MCT2 in rat brain and primary neuron cultures [53], remains to be investigated. Very recently in an astrocyte cancer cell line Lemire et al. showed good evidence of the presence of mL-LDH [54]. This further confirms that intracellular L-LAC shuttles could occur in both astrocytes and neurons.

1.6. Final remarks on L-LAC transport and metabolism in mammalian mitochondria

The central points that have been established in the studies reported in this minireview with respect to L-LAC transport and metabolism are as follows:

(i) L-LAC can enter mitochondria via a proton compensated symport and in addition L-LAC/PYR and L-LAC/OAA antiporters have been shown to exist [16]. All these L-LAC carriers are different from MPC, which is not a member of the MCT family [55]. On the other hand, some MCTs have also been found in mitochondria as immunologically investigated. Due to the absence of functional studies, the transport processes mediated by these MCTs remain only a matter of speculation.



Scheme 2. L-Lactate metabolism in brain: astrocyte–neuron shuttle, intracellular L-lactate shuttle and the L-lactate/pyruvate shuttle. The picture of L-LAC metabolism emerging from some papers quoted in this minireview is as follows. Astrocytes can produce L-LAC from taken up glucose. L-LAC can be oxidized inside mitochondria via the mL-LDH (Intracellular L-lactate shuttle) and/or released in the extracellular fluid from which it can be taken up by neurons (astrocyte/neuron shuttle). Inside neuron, L-LAC enters mitochondria via the L-LAC/H^+ symporter as well as in exchange with endogenous PYR which originates in the matrix via the mL-LDH. The newly synthesised PYR can in turn move in a carrier-mediated manner to the extramitochondrial phase where is converted to L-LAC (intracellular L-lactate/pyruvate shuttle). *Abbreviations*: IMS, intermembrane space; L-LAC, L-lactate; MIM, mitochondrial inner membrane; MOM, mitochondrial outer membrane, PM, plasma membrane; PYR, pyruvate; R.C., respiratory chain. Plasma membrane transporters: 1,2, astrocyte monocarboxylate transporters (MCT1, MCT4); 3, astrocyte and endothelial cell glucose transporter (GLUT1); 4, neuronal monocarboxylate transporter (MCT2); 5, neuronal glucose transporter (GLUT3). Mitochondria carriers: L-LAC, L-LAC/H^+ symporter; L-LAC/PYR, L-lactate/pyruvate antiporter; MPC, mitochondrial pyruvate translocator. Enzymes: cL-LDH, cytosolic L-lactate dehydrogenase; mL-LDH, mitochondrial L-lactate dehydrogenase.

It should be noted that both genomic and proteomic studies are of no help since in distinction with what occurs for L-LAC transport across the plasma membrane (see [56,57]), current database searching yields no detailed information on L-LAC

transport across the mitochondrial inner membrane. This is not surprising because even though many genes are reported to be mitochondrial carriers, most of them have no function assigned to them. Consistently mitochondrial carriers for relevant metabolites such as pyruvate, glutamine, choline etc whose transport activities have been extensively investigated in isolated intact mitochondria, await identification [58]. The clear lesson from this is that functional studies remain essential to improve knowledge of mitochondrial transport and metabolism.

(ii) The mL-LDH exists in mitochondria and is located in the inner mitochondrial compartment as clearly shown (a) by the experiments in which the fluorescence of the intramitochondrial pyridine nucleotides was found to increase as a result of L-LAC addition (b) by L-LDH activity assay carried out in solubilized mitochondria/mitochondrial fractions.

The puzzling situation of the "lactate oxidation complex" including mL-LDH, MCT1 and the cytochrome *c* oxidase (COX), remains to be elucidated; in fact the localization of mL-LDH in the outer side of the mitochondrial inner membrane as reported in skeletal muscle mitochondria [30,38] does not require L-LAC transport, thus the MCT1 should be assumed to transport the newly synthesised PYR (but see also above [55]). However, recently it was suggested that the neuron mitochondrial lactate oxidation complex contains a lactate/pyruvate transport protein (MCT1 or MCT2), mL-LDH and COX [53]. In this case, the occurrence of an L-LAC transporter in mitochondria is consistent with the occurrence of mL-LDH in the mitochondrial inner compartment shown in [52].

Finally the authors of this minireview believe that mL-LDH exists in the inner mitochondrial compartment where L-LAC is transported via some carriers.

2. Mitochondrial metabolism of L-lactate in plants

In distinction with mammalian cells, the knowledge of L-LAC metabolism in plants and in particular in mitochondria is very poor. However in plants, anoxic and more importantly hypoxic conditions lead to L-LAC accumulation in the cytosol with consequent decrease in pH and a switch in metabolism toward to ethanolic fermentation [59]. It was shown that in potato tubers, mitochondria play a major role in removal of L-LAC as a result of the occurrence of mL-LDH in the matrix to which L-LAC is transported in a carrier-mediated manner both via a proton compensated symporter and in antiport with PYR in the plant L-LAC/PYR shuttle [60]. Since plant mitochondria contain the alternative oxidase, which is activated by PYR [61], reducing equivalents from mitochondrial L-LAC can be transferred directly to oxygen, without proton ejection in the intermembrane space (Scheme 1). Thus such a shuttle possesses the unique characteristic of providing a non-energy-competent mechanism for the oxidation of cytosolic NADH [60]. Interestingly, these processes are only observed in mitochondria isolated from potato tubers not subjected to post harvest treatment. Mitochondria from potato tubers from the local market contained mL-LDH protein, as immunologically detected, but had no mL-LDH activity, this raising questions about the extent to which post harvest processes lead to metabolic changes in some plant systems [62]. The findings in [60,62] show the occurrence of the mitochondria L-LAC metabolism in plants. Whether and how this occurs in other plants remains to be established.

3. Mitochondrial metabolism of L-lactate in yeast

L-LAC is one of the major non-fermentable carbon and energy source used by yeast *Saccharomyces cerevisiae* under aerobic conditions of growth. The existence of a mitochondrial L-LAC metabolism has already been proposed in yeast (for ref. see [63]).

The processes by which metabolism of L-LAC occurs in yeast differ depending on the cell growth conditions. In cells grown anaerobically, an NAD-dependent mL-LDH was found to be induced both at high (3%) and low (0.6%) glucose concentration [64]. Both the intramitochondrial localization and the role of this enzyme in mitochondrial yeast metabolism remain to be further investigated. On the other hand, when L-LAC was used as a carbon source under aerobic conditions, a flavin mitochondrial L(+)-lactate: cytochrome *c* oxidoreductase was identified [65], encoded by the gene *CYB2* (*Cyb2p*) [66] and located in the intermembrane space [63]. *Cyb2p* oxidizes L-LAC directly with reduction of cytochrome *c* and final ATP generation (see [67] and refs. therein) (Scheme 1). Yeast mitochondria were found to swell in isoosmotic ammonium L-LAC solution [68] in a manner inhibited by non-penetrant compounds, this suggests the occurrence carrier-mediated transport of L-LAC into yeast mitochondria. Recently, the carrier which translocates PYR (MPC), has been identified in *Saccharomyces cerevisiae* mitochondria [17]. However, in the absence of functional studies, we cannot know whether and how MPC is involved in L-LAC transport by yeast mitochondria.

4. Conclusions

In the light of the findings reported in this minireview, L-LAC metabolism in mammalian, plant and yeast cells should be revised. In particular in mammalian it would be interesting to investigate whether and how the achievements outlined here for L-LAC mitochondrial metabolism apply to cancer in which L-LAC metabolism plays a major role and to some diseases including hypertension and diabetes.

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