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ORIGINAL PAPER

Twenty-seven Years of Cerebral Pyruvate Recycling

Sebastián Cerdán[1]

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Abstract Cerebral pyruvate recycling is a metabolic pathway deriving carbon skeletons and reducing equivalents from mitochondrial oxaloacetate and malate, to the synthesis of mitochondrial and cytosolic pyruvate, lactate and alanine. The pathway allows both, to provide the tricarboxylic acid cycle with pyruvate molecules produced from alternative substrates to glucose and, to generate reducing equivalents necessary for the operation of NADPH requiring processes. At the cellular level, pyruvate recycling involves the activity of malic enzyme, or the combined activities of phosphoenolpyruvate carboxykinase and pyruvate kinase, as well as of those transporters of the inner mitochondrial membrane exchanging the corresponding intermediates. Its cellular localization between the neuronal or astrocytic compartments of the in vivo brain has been controversial, with evidences favoring either a primarily neuronal or glial localizations, more recently accepted to occur in both environments. This review provides a brief history on the detection and characterization of the pathway, its relations with the early developments of cerebral high resolution ^{13}C NMR, and its potential neuroprotective functions under hypoglycemic conditions or ischemic redox stress.

Keywords Pyruvate recycling · ^{13}C NMR · ^{13}C isotopes · ^{13}C isotopomer analysis · Malic enzyme

Abbreviations

α-KG	α-ketoglutarate
Lac	Lactate
LDH	Lactate dehydrogenase
NMR	Nuclear magnetic resonance
ME	Malic enzyme (E.C. 1.1.1.40)
MRS	Magnetic resonance spectroscopy
Mal	Malate
OAA	Oxaloacetate
PDH	Pyruvate dehydrogenase (E.C. 1.2.4.1., 1.8.1.4., 2.3.4.12)
PEPCK	Phosphoenolpyruvate carboxykinase (E.C. 4.1.1.1.32)
PK	Pyruvate kinase (E.C. 2.7.1.40)
TCA	Tricarboxylic acid cycle

Introduction

I am honoured to contribute this short review on cerebral pyruvate recycling for the special issue of Neurochemical Research dedicated to the scientific trajectory of Prof. Ursula Sonnewald. Cerebral pyruvate recycling represented a fascinating aspect of the early activity of our laboratories and became the matter of many unforgettable conversations worldwide. This topic, initially restricted to few ^{13}C Nuclear Magnetic Resonance Spectroscopy (NMR) specialists, extended eventually to the neurochemical community and ultimately framed the applications of high resolution ^{13}C NMR to Neurochemistry. The following paragraphs summarize some of my personal recollections on the initial events leading to the detection of the pathway, the controversies that arose later on its cellular localization in the brain and some considerations on its possible functions.

Special Issue: Dedicated to Prof. Ursula Sonnewald.

✉ Sebastián Cerdán
scerdan@iib.uam.es

[1] Instituto de Investigaciones Biomédicas "Alberto Sols" CSIC/UAM, c/ Arturo Duperier 4, 28029 Madrid, Spain

^{13}C NMR studies of metabolism were pioneered by Eakin et al. when investigating, already in 1972, the metabolism of (1-^{13}C) glucose in yeast preparations [1]. The results obtained, made these authors envision that "^{13}C NMR could have numerous applications for in vivo metabolic studies". Indeed, ^{13}C NMR entailed important advantages that would make it later become the leading tool for metabolic studies [2]. Briefly, due to the low natural abundance of ^{13}C (1.1% ^{13}C), ^{13}C NMR provided a convenient method to detect the incorporation of the stable isotope ^{13}C, from suitable ^{13}C enriched substrates (99.9% ^{13}C) into metabolic intermediates and products. This wealth of information could be gathered not only in vitro, but even in vivo, making soon ^{13}C NMR to become the dominant technique in studies of cerebral metabolism [3, 4]. However, despite its important advantages, the ^{13}C NMR method was not devoid of limitations, its reduced sensitivity (in the mM range) representing, most probably, the largest. In fact, few scientists thought in the late 1980´s of the last century, that ^{13}C NMR could improve the results provided earlier by the highly sensitive radioactive isotope approach. This occurred even if ^{13}C NMR avoided the complexities of extraction, isolation and chemical degradation of the metabolites investigated, as imposed by adequate radioactive counting [5].

Even though its inherent sensitivity limitations, high resolution ^{13}C NMR entailed the unique advantage of enabling to explore the path of groups of contiguously enriched ^{13}C carbons, from a multiply labeled ^{13}C enriched substrate, into metabolic intermediates and products [6]. Notably, ^{13}C NMR could easily distinguish if a specific ^{13}C carbon within a metabolite molecule was not vicinal to any ^{13}C labeled neighbors, flanked by one ^{13}C, or two ^{13}C carbon atom neighbors [7]. Metabolites having the same chemical structure, but different distributions of ^{13}C atoms within their carbon skeleton, were named "isotopic isomers" or "isotopomers". Different "isotopomers" originated characteristic homonuclear ^{13}C–^{13}C spin coupling patterns in the ^{13}C resonances from every carbon, revealing the relative isotopomer contributions after appropriate spectral analysis. This exceptional advantage became later instrumental for the applications of high resolution ^{13}C NMR to neurochemistry in general and, for the detection of the cerebral pyruvate recycling system, in particular.

Cerebral Pyruvate Recycling In Vivo

Early 1989, during a postdoctoral period in Basel (CH), we explored the cerebral metabolism of the simplest molecule containing two contiguously ^{13}C enriched carbons, namely $^{13}C_2$ acetate [8]. We infused $^{13}C_2$ acetate in the right jugular vein of fed rats and monitored, in vivo and in vitro, the fate of the two vicinally ^{13}C enriched carbons. In vivo ^{13}C NMR spectroscopy was not sufficiently developed at the time, and we could only detect in vivo, resonances from the infused substrate and ^{13}C bicarbonate. However, the analysis of ^{13}C resonances from glutamate and glutamine carbons in perchloric acid extracts obtained from the brain after $^{13}C_2$ acetate infusion, became much more informative. Indeed, high resolution ^{13}C NMR multiplet analysis of brain extracts yielded a wealth of metabolic information on cerebral metabolism and provided the first evidence of cerebral pyruvate recycling.

Figure 1a shows an expanded view of the ^{13}C resonances from the C4 carbons of cerebral glutamate (resonance #9) and glutamine (resonance #8) in these extracts, and their deconvolution into the corresponding contributions of (4-^{13}C), (4,5-$^{13}C_2$), and (3,4,5-$^{13}C_3$) isotopomers, represented by the singlet (Fig. 1b), doublet (Fig. 1c), and doublet of doublets (Fig. 1d) resonances, respectively. First, ^{13}C incorporation was larger in glutamine C4 than in the glutamate C4 carbons, as reflected by the larger intensity of the glutamine resonances. This result was inconsistent with the strict precursor-product relationships, precluding the glutamine product to incorporate more ^{13}C label than its glutamate precursor. The observation confirmed previous reports on the metabolism of ^{14}C acetate, revealing the presence of "large" and "small" glutamate pools and the predominant labelling of the "small" glutamate pool and glutamine by ^{14}C acetate [9–11]. The finding was not new then, but became very relevant for further studies, since it validated the recent ^{13}C NMR methodology with earlier ^{14}C results, proving that both approaches yielded similar outcomes. Indeed, the "small" and "large" glutamate pools had been reported to reflect metabolism in the glial and neuronal compartments, respectively [10, 12, 13]. The "small" glutamate pool, was associated with a "fast turnover" tricarboxylic acid (TCA) cycle, thought to occur in the glial compartment and generating most of the cerebral glutamine, a finding later supported by reports showing the predominant acetate transport to glial cells [14]. Conversely, the "large" glutamate pool was associated with a "slow turnover" TCA cycle occurring in the neuronal compartment and originating the largest proportion of cerebral glutamate [10]. It was then very surprising, to find that a high resolution ^{13}C NMR spectrum from a rat brain extract, obtained after infusion with $^{13}C_2$ acetate, could include enough information to summarize decades of previous research with radioactive isotopes, a finding supporting further applications of the ^{13}C NMR method.

The C4 and C5 resonances of glutamate and glutamine in particular, became highly informative, since they reflected directly the ^{13}C isotopomer distributions of the C2 and C1 carbons of acetyl-CoA molecules, entering the neuronal or glial tricarboxylic acid cycles, respectively.

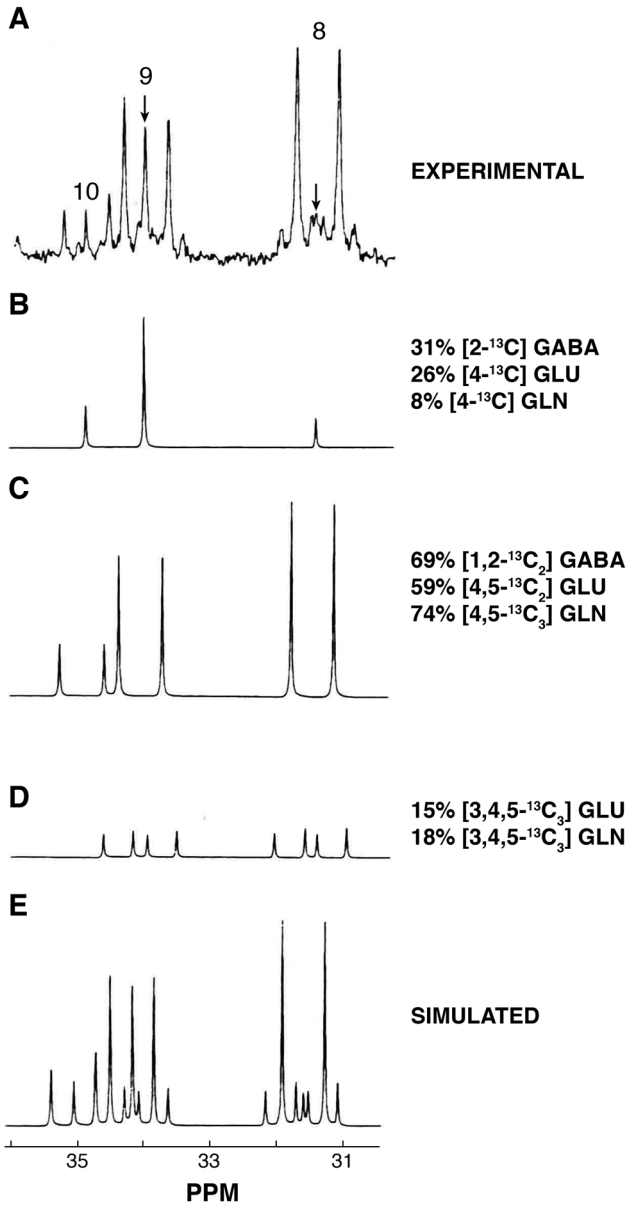

Fig. 1 Analysis of the ^{13}C labelling patterns of the C4 carbons from glutamate (resonance #9) and glutamine (resonance #8) from brain extracts obtained after (1,2-$^{13}C_2$) acetate ^{infusion}. **a** Experimental spectrum, **b** relative contributions of (4-^{13}C) glutamate and (4-^{13}C) glutamine to the corresponding C4 multiplets, **c** relative contributions of (4,5,-$^{13}C_2$) glutamate and glutamine, **d** relative contribution of (3,4,5-$^{13}C_3$) glutamate and glutamine, **e** simulated spectrum. Reproduced from ref. [8] with permission

We were amazed to find, in addition to the expected glutamate and glutamine C4 doublet resonances derived directly from infused (1,2-$^{13}C_2$) acetate (Fig. 1a), a central singlet (full arrow), derived from monolabeled (4-^{13}C) glutamate and (4-^{13}C) glutamine isotopomers. Similar ^{13}C multiplet patterns were found in the C5 carbons of glutamate and glutamine, as expected (not shown). Notably, the central singlets in glutamate and glutamine C4 and C5 resonances revealed that (2-^{13}C) and (1-^{13}C) acetyl-CoA had been produced during oxidative metabolism of $^{13}C_2$ acetate, reentering later the TCA cycle. The mechanism by which these monolabeled forms of acetyl-CoA were originated from $^{13}C_2$ acetate, disclosed the presence of the cerebral pyruvate recycling system. Since we (and others) did not observe monolabeled forms of acetate in plasma during metabolism of $^{13}C_2$ acetate in fed rats or humans, we concluded that the monolabeled forms of acetate were originated mainly in the brain [8, 15]. Indeed, monolabeled (1-^{13}C) or (2-^{13}C) acetyl-CoA, could be produced either from (1,2-$^{13}C_2$) and (3-4-$^{13}C_2$) oxaloacetate or from (1,2-$^{13}C_2$) and (3-4-$^{13}C_2$) malate, through phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate kinase (PK), or malic enzyme (ME) activities, respectively. The relative contribution of these enzymes will be addressed later.

Further analysis of the glutamate and glutamine C4 multiplets, provided information on the compartmentation of the pyruvate recycling pathway. Figure 1b–e show the complete deconvolution of the glutamate and glutamine C4 multiplets, in the contributions of individual C4 resonances from the corresponding (4-^{13}C) (Fig. 1b), (4,5-$^{13}C_2$) (Fig. 1c) and (3,4,5-$^{13}C_3$) isotopomers (Fig. 1d). A simulated addition of all contributions (Fig. 1e) shows remarkable similarity with the experimental spectrum (Fig. 1a), supporting an adequate deconvolution. Notably, the relative contribution of (4-^{13}C) glutamate (29%) was much larger than that of (4-^{13}C) glutamine (8%). This finding revealed that the pyruvate recycling was mainly located in a compartment with lower (or absent) glutamine synthase activity. Otherwise the relative contributions of (4-^{13}C) isotopomers in glutamate and glutamine should be equal, in an non-compartmented system with a homogenous distribution of the enzyme. Since glutamine synthase was shown to be exclusively located in the glial compartment [16], we proposed that the monolabeled forms of acetyl-CoA and therefore the pyruvate recycling system was mainly located in the "large" glutamate compartment, a circumstance accounting for the reduced (or absent) formation of (4-^{13}C) glutamine from (4-^{13}C) glutamate (Fig. 2a). In fact, it became possible later to demonstrate the preferential location of the enzymes involved in the synaptosomal fraction obtained by centrifugal fractionation of adult brain homogenates in Percoll density gradients (Fig. 2b) [17].

We investigated later if the cerebral pyruvate recycling system was active with other infused ketogenic or gluconeogenic substrates, as (U-$^{13}C_4$) hydroxybutyrate or (1,2-$^{13}C_2$) glucose [18]. The glutamate C4 and C5 multiplets of glutamate and glutamine observed in acid extracts of the brain after these infusions, depicted similar structures to those depicted in Fig. 1, in particular the singlets (Fig. 1b). Notably, both precursors yielded (1,2-$^{13}C_2$) acetyl-CoA and its

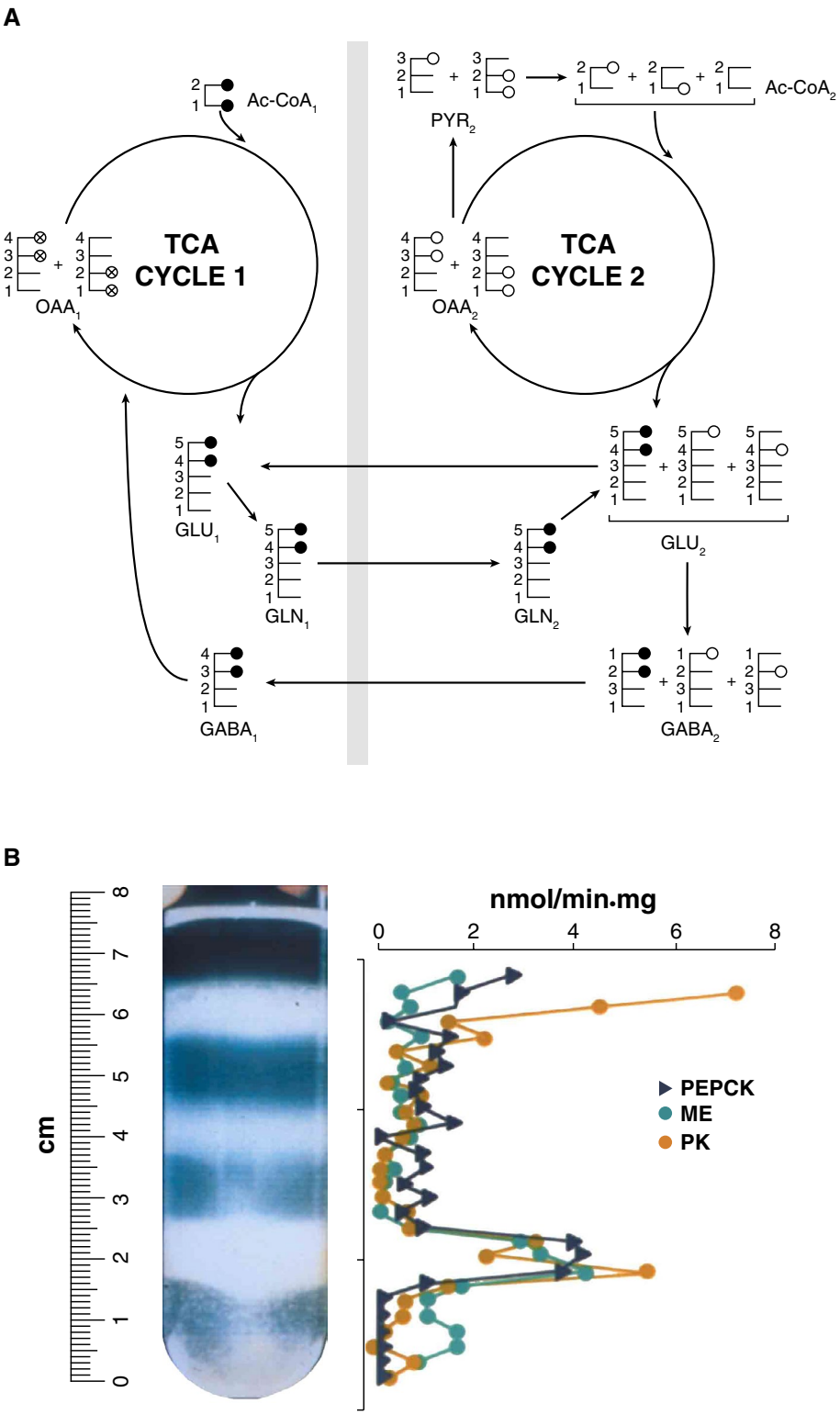

Fig. 2 Early evidences on the compartmentation of the cerebral pyruvate recycling in the adult rat brain. **a** Cerebral tricarboxylic acid cycles of the neuronal (TCA cycle 2) and glial (TCA cycle 1) compartments during $^{13}C_2$ acetate metabolism. The glial cycle metabolizes $^{13}C_2$ acetate (*black filled circles*), while the neuronal cycle metabolizes mainly unlabeled acetyl-CoA (*open circles*). Glutamate, glutamine and GABA are exchanged between both TCA cycles. The different symbols (*crossed circles* and *open circles*) in OAA1 and OAA2 indicate that different ^{13}C enrichments may occur. The absence of glutamine synthase in the neuronal compartment, precludes the synthesis of neuronal glutamine from neuronal glutamate. This accounts for the smaller contribution of monolabeled forms of acetate to glutamine labelling (Fig. 1a–b), suggesting a dominant contribution of the recycling pathway in the neuronal TCA cycle 2. Reproduced from ref [8] with permission. **b** Synaptosomal localization of the enzymatic activities involved in the recycling of pyruvate in the adult rat brain in a Percoll density gradient (Cruz, F. and Cerdán, S., unpublished observations). *ME* malic enzyme, *PK* pyruvate kinase, *PEPCK* phosphoenolpyruvate carboxykinase

recycled (1-^{13}C) and (2-^{13}C) acetyl-CoA isotopomers, indicating that recycling of (1,2-$^{13}C_2$) acetyl-CoA units, was not limited to $^{13}C_2$ acetate. However, the relative contribution of the pyruvate recycling system to acetyl-CoA oxidation was smaller with (U-^{13}C)-hydroxybutyrate (17%) or with (1,2-$^{13}C_2$) glucose (5%) than with $^{13}C_2$ acetate (27%), suggesting that the ketogenic or gluconeogenic nature of the precursor, had an important influence on the relative contribution of the recycling pathway to acetyl-CoA oxidation. More oxidized substrates as $^{13}C_2$ acetate or (U-$^{13}C_4$)

β-hydroxybutyrate, depicted larger relative contributions of recycled, monolabeled forms of acetate, than more reduced substrates as (1,2-$^{13}C_2$) glucose. This suggested that the intracellular redox state of the brain, as determined by the substrate infused, played an important role in the regulation of the pyruvate recycling pathway.

Interestingly, both $^{13}C_2$ acetate and (U-$^{13}C_4$) hydroxybutyrate originated small amounts of (2,3-$^{13}C_2$) lactate, derived from (3,4-$^{13}C_2$) oxaloacetate and malate, through PEPCK and PK or malic enzyme activities, suggesting that the (2,3-$^{13}C_2$) pyruvate produced intramitochondrially by these reactions, was transported into the cytosol, becoming the substrate for lactate dehydrogenase [18–21]. This finding indicated that recycling was not limited to the mitochondrial compartment hosting the TCA cycle, but disclosed an additional cytosolic component resulting in recycled lactate production.

The precise mechanism of the recycling remained uncertain, since different enzymes as PEPCK and PK or ME, could originate from the same ^{13}C labelling pattern in the C4 (and C5) carbons of glutamate and glutamine. To overcome this difficulty, we designed an independent approach comparing the developmental profiles of these enzymes to that of the recycling pathway [17]. We measured by ^{13}C NMR the change in relative pyruvate recycling activity in the brain of rats with increasing ages, from postnatal day 0 (P0) to postnatal day 90 (P90, Fig. 3a–b), comparing it with the developmental profile of the enzymatic activities of PEPCK (Fig. 3c), ME (Fig. 3d) and PK (Fig. 3e). The pyruvate recycling system developed in parallel with myelination, peaking at day P21 and maintaining the same activation until day P90. While PEPCK and PK maintained similar activities along development, ME activity increased in parallel to the activity of the pyruvate recycling system, suggesting that ME contributed the dominant enzymatic mechanism of this pathway.

The localization of malic enzyme in brain had been extensively investigated at the time, by the Hamprecht´s and McKenna´s groups [22–24]. Brain homogenates contained 45% of the cytosolic enzyme (ME_c), while astroglial cultures contained 95% of ME_c, suggesting that the cytosolic enzyme was primarily located in astrocytes [23]. In contrast, inmunocytochemical staining of rat brain sections with antibodies prepared against the mitochondrial enzyme (ME_m), stained primarily the neurons [22]. Moreover, ME activity was much higher in mitochondria from synaptic terminals, than in mitochondria prepared from primary cultures of cortical neurons or cerebellar granule cells [24]. In summary, both neurons and glial cells were equipped with malic enzyme, albeit with different subcellular

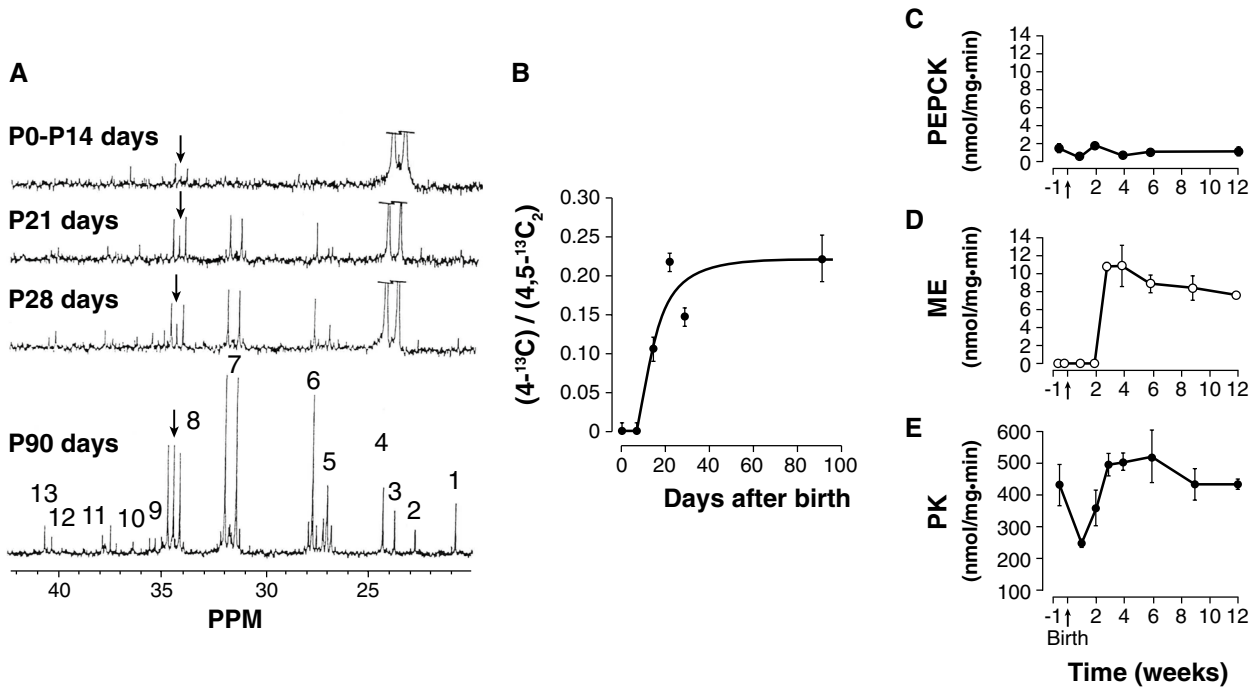

Fig. 3 Cerebral pyruvate recycling after infusion of $^{13}C_2$ acetate in rats of increasing ages P0-P90. **a** High resolution ^{13}C NMR spectra of acid extracts from rat brain during development after administration of (1,2-$^{13}C_2$) acetate. *Arrow* Glutamate C4 singlet revealing the relative contribution recycled (2-^{13}C) acetyl-CoA. **b** Developmental changes in the relative activity of the cerebral pyruvate recycling system as detected in the singlet/doublet ratio of Glutamate C4, **c–e** Developmental changes in the enzymatic activities of PK, ME and PEPCK. Reproduced from ref. [17] with permission

localizations, the mitochondrial enzyme from neurons providing a slightly larger contribution to total malic enzyme activity in the adult rat brain. This subcellular distribution of malic enzymes matched well the cytosolic and mitochondrial components of the pyruvate recycling system detected in vivo, suggesting that mitochondrial ME_m present in neurons could originate the mitochondrial pyruvate recycling, while the cytosolic ME_c could provide the cytosolic recycling. However, it remained to be explained why the recycling detected in vivo during the metabolism of $^{13}C_2$ acetate was much smaller in glutamine than in glutamate, representing the glial and neuronal compartments, since the total enzymatic activities of ME in these compartments were relatively similar, as measured by enzymatic methods.

We thought then that the relative sizes of the neuronal and glial pools of glutamate and glutamine, could underlie a differential sensitivity of ^{13}C NMR to detect the neuronal or glial metabolisms of $^{13}C_2$ acetate in vivo. Traditionally, the determination of the pool sizes of the neuronal and glial glutamate pools in vivo had remained very challenging, normally approached by mathematical modelling and immunocytochemistry [10, 12, 25, 26]. To tackle this issue experimentally, we implemented a novel strategy using both, the ^{13}C isotope and an additional isotope, namely deuterium [27]. We infused either solutions of (2-^{13}C, 2-$^{2}H_3$) acetate or mixtures of (2-^{13}C, 2-$^{2}H_3$) acetate and (1-^{13}C) glucose in the right jugular vein of rats. The presence of ^{13}C-^{2}H coupling patterns, only in those acetyl-CoA molecules derived from deuterated acetate, made it possible to distinguish clearly between the (2-^{13}C, 2-$^{2}H_3$) acetyl-CoA molecules derived from deuterated acetate, and the (2-^{13}C) acetyl CoA molecules derived from non-deuterated glucose. This allowed for the direct determination of the neuronal and glial pool sizes of glutamate, by solving a simple set of simultaneous equations involving mass and ^{13}C enrichment conservation. We found that the neuronal and glial pool sizes of glutamate, were drastically different during (2-^{13}C, 2-$^{2}H_3$) acetate or acetate + (1-^{13}C) glucose infusions. Notably, the glial pool size of glutamate increased from 8.6 ± 2% during glucose and acetate infusions, to 73 ± 8% during acetate infusions, suggesting that glial processes affecting the glutamate C4 resonances would become much more difficult to detect with mixtures of glucose and acetate than with acetate as the only substrate. However, even under these unfavorable conditions, we were able to measure a small contribution of glial pyruvate recycling by detecting recycled (4-^{13}C, 4-^{2}H) glutamine, indicating that both neuronal and astrocytic pyruvate recycling systems could be detected in vivo with this approach. Scafidi et al. [28] confirmed these findings by detecting metabolism of the acetyl moiety from acetylcarnitine (ALCAR) both in astrocytes and GABAergic neurons.

Serres et al. [29] investigated later the effects of metabolism of mixtures of (2-^{13}C) acetate and glucose and acetate and (1-^{13}C) glucose, in awake or anesthetized rats, using proton observed ^{13}C decoupled spectroscopy (POCE). Briefly, they found that different anesthetics did not induce important alterations in the activity of the pyruvate recycling system. Additionally, ^{13}C fractional enrichments of the protonated carbons of glutamate and glutamine, as measured in the brain, were lower than those of their precursors measured in plasma. On these grounds, these authors proposed that recycled isotopomers of pyruvate in the brain could be mainly derived from peripheral tissues. However, the precise contribution of intracerebral substrates could not be established and POCE did not allow the analysis of the relative contributions of non-protonated glutamate isotopomers. Therefore, a possible underestimation of the contribution of intracerebral recycling could not be discarded. In spite of this, the experiments provided a relevant note of caution, suggesting that the contribution of peripheral tissues, to the pyruvate recycling system observed in the in vivo brain, could be larger than previously thought.

Cerebral Pyruvate Recycling Ex Vivo

The Sonnewald´s and Schouboe´s groups had implemented an extensive collection of experiments to characterize cerebral pyruvate recycling both in vivo, and in vitro, using primary cultures of neural cells enriched in neurons or astrocytes [19, 20, 30–35]. The pyruvate recycling pathway was detected initially in primary cultures of astrocytes but not in neurons [20, 32]. Briefly, astrocytes cultures incubated with (U-$^{13}C_5$) glutamine, showed the presence of (4,5-$^{13}C_2$) glutamate, an isotopomer requiring the formation of (U-$^{13}C_3$) pyruvate and ($^{13}C_2$)-acetyl-CoA and its incorporation into unlabeled oxaloacetate in the glial TCA cycle [19]. Subsequent experiments used (3-^{13}C) glutamate incubations with primary cultures of cerebrocortical astrocytes, GABAergic cerebrocortical interneurons and co-cultures of both cell types [32]. (4-^{13}C) glutamate derived from recycling could only be detected in astrocyte cultures, suggesting that cultivated astrocytes, but not cultivated neurons, were endowed with the pyruvate recycling capacity. Lastly, pyruvate recycling could be demonstrated, both by gas chromatography-mass spectrometry and ^{13}C NMR, in primary cultures enriched in cerebellar neurons, confirming that the recycling pathway was active both in cell cultures from neurons and astrocytes, as well as in vivo [31], a finding consistent with the presence of mitochondrial and cytosolic malic enzymes in neurons and astrocytes, respectively [22–24].

Relevance of Cerebral Pyruvate Recycling

An important aspect relates to the physiological functions of cerebral pyruvate recycling. First, recycled carbons from substrates other than glucose, may provide a useful alternative to maintain operative the vital TCA cycle when glucose supplies are limited. This has been shown to occur under ischemic conditions, where glucose supplies to the infarcted regions decrease, and the contribution of cerebral pyruvate recycling increases, using either glutamate or glutamine as substrates [36, 37]. Second, NADPH production by malic enzyme activity might be essential for fatty acid synthesis during physiological myelin formation and thus, become a target to support remyelinating therapies [38, 39]. Third, increased NADPH availability may become very useful in neuroprotection, modulating the GSH/GSSG ratios to protect from free radical damage and oxidative stress [40]. Finally, pyruvate recycling may underlie the neuroprotective effects of ketogenic diets in several neurodegenerative disorders [41–43]. It is also possible that neuronal and glial recycling pathways serve different cerebral functions. In fact, mitochondrial and cytosolic isoenzymes of ME could underlie important cerebral processes as redox regulation and neuroprotection or fatty acid synthesis and myelination, respectively.

To conclude, the pyruvate recycling system in the brain has come a long way since its detection in 1990. It has been demonstrated to occur in the neuronal and glial compartments, both in vivo and in vitro, with potentially important functions. However, uncertainties still remain on the relative contributions of the neuronal and glial systems to the overall process as observed in vivo, or on the precise contribution of peripheral tissues to the recycling processes detected in brain under different conditions. These aspects deserve further exploration. However, the fact that the pyruvate recycling pathway appears to occur ubiquitously in the brain, strongly suggests that it may play a vital role supporting adequate cerebral function.

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References

1. Eakin RT, Morgan LO, Gregg CT, Matwiyoff NA (1972) Carbon-13 nuclear magnetic resonance spectroscopy of living cells and their metabolism of a specifically labeled 13C substrate. FEBS Lett 28:259–264
2. Rodrigues TB, Valette J, Bouzier-Sore AK (2013) (13)C NMR spectroscopy applications to brain energy metabolism. Front Neuroenerg 5:1–16
3. Shulman RG, Rothman DL (2001) 13C NMR of intermediary metabolism: implications for systemic physiology. Annu Rev Physiol 63:15–48
4. Shulman RG, Rothman DL (eds) (2005) Metabolomics by in vivo NMR. Wiley, Chichester, UK
5. Cerdan S, Seelig J (1990) NMR studies of metabolism. Ann Rev Biophys Biophys Chem 19:43–67
6. Kunnecke B, Cerdan S (1989) Multilabeled 13C substrates as probes in in vivo 13C and 1H NMR spectroscopy. NMR Biomed 2:274–277
7. Rodrigues TB, Cerdán S (2005) ^{13}C NMR: an outstanding tool for metabolic studies. Concepts Magn Reson Part A 27A(1):1–16
8. Cerdan S, Kunnecke B, Seelig J (1990) Cerebral metabolism of [1,2-13C2]acetate as detected by in vivo and in vitro 13C NMR. J Biol Chem 265:12916–12926
9. Rodrigues TB, Cerdán S (2007) The Cerebral Tricarboxylic Acid ycles. In: Lajhta A (ed) Handbook of neurochemistry and molecular neurobiology:brain energetics, integration of molecular and cellular processes, 3rd edn. Springer, New York, pp 485
10. van den Berg CJ, Garfinkel D (1971) A simulation study of brain compartments. Metabolism of glutamate and related substances in mouse brain. Biochem J 123:211–218
11. Berls S, Clarke DD (1969) Compartmentation of aminoacid metabolism. Plenum Press, New York
12. Garfinkel D (1966) A simulation study of the metabolism and compartmentation in brain of glutamate, aspartate, the Krebs cycle, and related metabolites. J Biol Chem 241:3918–3929
13. Garfinkel D (1970) A simulation study of brain compartments. I. Fuel sources, and GABA metabolism. Brain Res 23:387–406
14. Waniewski RA, Martin DL (1998) Preferential utilization of acetate by astrocytes is attributable to transport. J Neurosci 18:5225–5233
15. Lebon V, Petersen KF, Cline GW, Shen J, Mason GF, Dufour S, Behar KL, Shulman GI, Rothman DL (2002) Astroglial contribution to brain energy metabolism in humans revealed by 13C nuclear magnetic resonance spectroscopy: elucidation of the dominant pathway for neurotransmitter glutamate repletion and measurement of astrocytic oxidative metabolism. J Neurosci 22:1523–1531
16. Martinez-Hernandez A, Bell KP, Norenberg MD (1977) Glutamine synthetase: glial localization in brain. Science 195:1356–1358
17. Cruz F, Scott SR, Barroso I, Santisteban P, Cerdan S (1998) Ontogeny and cellular localization of the pyruvate recycling system in rat brain. J Neurochem 70:2613–2619
18. Kunnecke B, Cerdan S, Seelig J (1993) Cerebral metabolism of [1,2-13C2]glucose and [U-13C4]3-hydroxybutyrate in rat brain as detected by 13C NMR spectroscopy. NMR Biomed 6:264–277
19. Sonnewald U, Westergaard N, Jones P, Taylor A, Bachelard HS, Schousboe A (1996) Metabolism of [U-13C5] glutamine in cultured astrocytes studied by NMR spectroscopy: first evidence of astrocytic pyruvate recycling. J Neurochem 67:2566–2572
20. Hassel B, Sonnewald U (1995) Glial formation of pyruvate and lactate from TCA cycle intermediates: implications for the inactivation of transmitter amino acids? J Neurochem 65:2227–2234

21. Rodrigues TB, Sierra A, Ballesteros P, Cerdán S (2011) Pyruvate Transport and Metabolism in the Central Nervous System. In: Choi I-Y, Gruetter R (eds) Neural metabolism in vivo (advances in neurobiology 4), Springer, New York, pp 715–753
22. Vogel R, Jennemann G, Seitz J, Wiesinger H, Hamprecht B (1998) Mitochondrial malic enzyme: purification from bovine brain, generation of an antiserum, and immunocytochemical localization in neurons of rat brain. J Neurochem 71:844–852
23. Vogel R, Hamprecht B, Wiesinger H (1998) Malic enzyme isoforms in astrocytes: comparative study on activities in rat brain tissue and astroglia-rich primary cultures. Neurosci Lett 247:123–126
24. McKenna MC, Stevenson JH, Huang X, Tildon JT, Zielke CL, Hopkins IB (2000) Mitochondrial malic enzyme activity is much higher in mitochondria from cortical synaptic terminals compared with mitochondria from primary cultures of cortical neurons or cerebellar granule cells. Neurochem Int 36:451–459
25. Ottersen OP, Storm-Mathisen J, Bramham C, Torp R, Laake J, Gundersen V (1990) A quantitative electron microscopic immunocytochemical study of the distribution and synaptic handling of glutamate in rat hippocampus. Prog Brain Res 83:99–114
26. Storm-Mathisen J, Leknes AK, Bore AT, Vaaland JL, Edminson P, Haug FM, Ottersen OP (1983) First visualization of glutamate and GABA in neurones by immunocytochemistry. Nature 301:517–520
27. Chapa F, Cruz F, Garcia-Martin ML, Garcia-Espinosa MA, Cerdan S (2000) Metabolism of (1-(13)C) glucose and (2-(13)C, 2-(2)H(3)) acetate in the neuronal and glial compartments of the adult rat brain as detected by [(13)C, (2)H] NMR spectroscopy. Neurochem Int 37:217–228
28. Scafidi S, Fiskum G, Lindauer SL, Bamford P, Shi D, Hopkins I, McKenna MC (2010) Metabolism of acetyl-L-carnitine for energy and neurotransmitter synthesis in the immature rat brain. J Neurochem 114:820–831
29. Serres S, Bezancon E, Franconi JM, Merle M (2007) Brain pyruvate recycling and peripheral metabolism: an NMR analysis ex vivo of acetate and glucose metabolism in the rat. J Neurochem 101:1428–1440
30. Amaral AI, Teixeira AP, Sonnewald U, Alves PM (2011) Estimation of intracellular fluxes in cerebellar neurons after hypoglycemia: importance of the pyruvate recycling pathway and glutamine oxidation. J Neurosci Res 89:700–710
31. Olstad E, Olsen GM, Qu H, Sonnewald U (2007) Pyruvate recycling in cultured neurons from cerebellum. J Neurosci Res 85:3318–3325
32. Waagepetersen HS, Qu H, Hertz L, Sonnewald U, Schousboe A (2002) Demonstration of pyruvate recycling in primary cultures of neocortical astrocytes but not in neurons. Neurochem Res 27:1431–1437
33. Haberg A, Qu H, Bakken IJ, Sande LM, White LR, Haraldseth O, Unsgard G, Aasly J, Sonnewald U (1998) In vitro and ex vivo 13C-NMR spectroscopy studies of pyruvate recycling in brain. Dev Neurosci 20:389–398
34. Sonnewald U, Westergaard N, Hassel B, Muller TB, Unsgard G, Fonnum F, Hertz L, Schousboe A, Petersen SB (1993) NMR spectroscopic studies of 13C acetate and 13C glucose metabolism in neocortical astrocytes: evidence for mitochondrial heterogeneity. Dev Neurosci 15:351–358
35. Hassel B, Sonnewald U, Fonnum F (1995) Glial-neuronal interactions as studied by cerebral metabolism of [2-13C]acetate and [1-13C]glucose: an ex vivo 13C NMR spectroscopic study. J Neurochem 64:2773–2782
36. Pascual JM, Carceller F, Roda JM, Cerdan S (1998) Glutamate, glutamine, and GABA as substrates for the neuronal and glial compartments after focal cerebral ischemia in rats. Stroke 29:1048–1056; **discussion 1056–1047**
37. Morken TS, Brekke E, Haberg A, Wideroe M, Brubakk AM, Sonnewald U (2014) Altered astrocyte-neuronal interactions after hypoxia-ischemia in the neonatal brain in female and male rats. Stroke 45:2777–2785
38. Harlow DE, Honce JM, Miravalle AA (2015) Remyelination Therapy in Multiple Sclerosis. Front Neurol 6:257
39. Franklin RJ, Ffrench-Constant C (2008) Remyelination in the CNS: from biology to therapy. Nature Rev Neurosci 9:839–855
40. Calabrese V, Cornelius C, Mancuso C, Lentile R, Stella AM, Butterfield DA (2010) Redox homeostasis and cellular stress response in aging and neurodegeneration. Methods Mol Biol 610:285–308
41. Shaafi S, Mahmoudi J, Pashapour A, Farhoudi M, Sadigh-Eteghad S, Akbari H (2014) Ketogenic diet provides neuroprotective effects against ischemic stroke neuronal damages. Adv Pharm Bull 4:479–481
42. Noh HS, Kim YS, Choi WS (2008) Neuroprotective effects of the ketogenic diet. Epilepsia 49(Suppl 8):120–123
43. Ruiz F, Alvarez G, Pereira R, Hernandez M, Villalba M, Cruz F, Cerdan S, Bogonez E, Satrustegui J (1998) Protection by pyruvate and malate against glutamate-mediated neurotoxicity. Neuroreport 9:1277–1282