REVIEW ARTICLE

Metabolic control exerted by the 2-oxoglutarate dehydrogenase reaction: a cross-kingdom comparison of the crossroad between energy production and nitrogen assimilation

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Mechanism-based inhibitors and both forward and reverse genetics have proved to be essential tools in revealing roles for specific enzymatic processes in cellular function. Here, we review experimental studies aimed at assessing the impact of OG (2-oxoglutarate) oxidative decarboxylation on basic cellular activities in a number of biological systems. After summarizing the catalytic and regulatory properties of the OGDHC (OG dehydrogenase complex), we describe the evidence that has been accrued on its cellular role. We demonstrate an essential role of this enzyme in metabolic control in a wide range of organisms. Targeting this enzyme in different cells and tissues, mainly by its specific inhibitors, effects changes in a number of basic functions, such as mitochondrial potential, tissue respiration, ROS (reactive oxygen species) production, nitrogen metabolism, glutamate signalling and survival, supporting the notion that the evolutionary conserved reaction of OG degradation is required for metabolic adaptation. In particular, regulation of OGDHC under stress conditions may be essential to overcome glutamate excitotoxicity in neurons or affect the wound response in plants. Thus, apart from its role in producing energy, the flux through OGDHC significantly affects nitrogen assimilation and amino acid metabolism, whereas the side reactions of OGDHC, such as ROS production and the carboligase reaction, have biological functions in signalling and glyoxylate utilization. Our current view on the role of OGDHC reaction in various processes within complex biological systems allows us a far greater fundamental understanding of metabolic regulation and also opens up new opportunities for us to address both biotechnological and medical challenges.

Key words: γ-aminobutyric acid (GABA), excitotoxicity, metabolic control, nitrogen assimilation, 2-oxoglutarate dehydrogenase (OGDH), thiamine deficiency.

INTRODUCTION

A current challenge in systems biology is to understand living systems to an extent which would allow us to control and/or change metabolism in a cell, tissue and organism according to our own design [1–3]. Theoretical and experimental studies performed to date indicate that the impact of particular metabolic reactions on general metabolic activity is far from equal. For example, Metabolic Control Analysis studies have indicated that, whereas pathway control is generally shared between several enzymes, the distribution of this control is not equivalent across the pathway and may vary depending on the conditions [4–6]. Other studies have revealed that only a limited amount of metabolic genes are essential for early embryonic development in zebrafish (*Danio rerio*) [7]. Furthermore, within central metabolic networks there are enzymatic processes whose perturbation greatly affects general performance, and those in which changes are easily accommodated without an obvious effect on basic functions. This is in accordance with results from a study of the global organization of metabolic fluxes in *Escherichia coli* in revealing that general metabolic activities are dominated by a limited number of reactions, which belong to a so-called 'high-flux backbone' of metabolism [8]. It is therefore especially interesting to be able to specifically manipulate the fluxes through these reactions *in vivo* in order to exert metabolic control.

A number of studies have pointed to an essential role of OGDHC (2-oxoglutarate dehydrogenase complex) in overall metabolic activity. Oxidative decarboxylation of OG (2-oxoglutarate) performed by the complex belongs to the 'high-flux backbone' of metabolism [8], and the genes for the complex components were shown to be essential for the early embryonic development in animals [7,9]. A crucial role of the OGDHC reaction is in keeping with the rare incidence in humans of the mutations of the OGDHC components. Even a partial impairment of the mutated OGDHC in humans results in neurological symptoms and early death [10–13]. On the other hand, age-related neurodegenerative diseases are also associated with a decrease in the OGDHC activity [14,15]. Thus the metabolic branch point of OG, which is either irreversibly degraded by OGDHC or provides carbon skeletons for nitrogen assimilation, is clearly of systemic importance. This is further supported by the rich regulation of OGDHC including allosteric responses to second messengers and metabolic indicators, such as Ca^{2+}, ATP/ADP, SH/-S-S (thiol/disulfide), NADH/NAD^+, acyl-CoA/CoA [16]

Abbreviations used: ArcA(B), aerobic respiratory control two-component system; CESP, carboxyethyl succinyl phosphonate; DESP, diethyl succinyl phosphonate; FNR, ferric nitrite reductase; GABA, γ-aminobutyric acid; NtcA, a global transcription factor of nitrogen and carbon metabolism belonging to the CRP (cAMP receptor protein) family; OG, 2-oxoglutarate; OGDH, 2-oxoglutarate dehydrogenase; OGDHC, oxoglutarate dehydrogenase complex; PESP, phosphonoethyl succinyl phosphonate; ROS, reactive oxygen species; SH/-S-S, thiol/disulfide; SP, succinyl phosphonate; TESP, triethyl succinyl phosphonate; ThDP, thiamine diphosphate.

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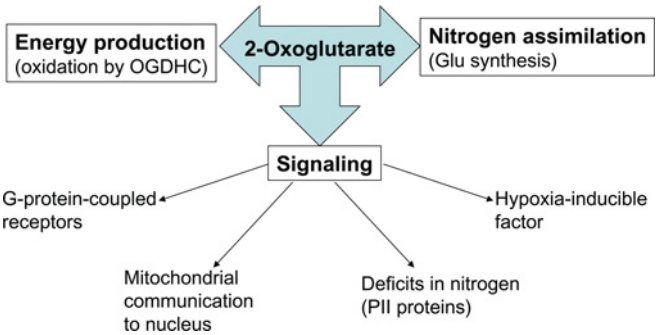


Figure 1 The diverse roles of OG

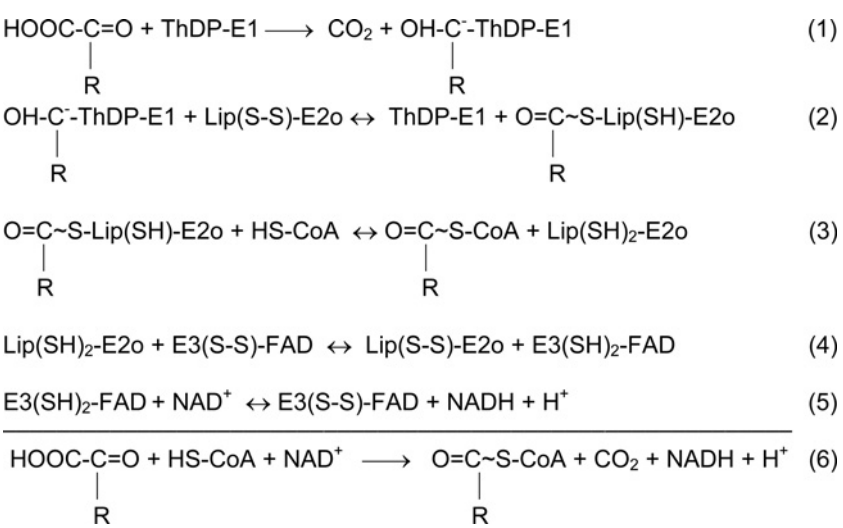
and the existence of multiple signalling mechanisms involving the OGDHC-degraded OG (Figure 1). These mechanisms include protein mediators, such as G-protein-coupled receptors involved in stimulating inositol phosphate synthesis [17] or the hypoxia-inducible factor prolyl hydroxylase [18,19]. Cellular sensing of the OG/glutamate ratio is, furthermore, at least in non-photosynthetic species, involved in retrograde signalling of mitochondria to the nucleus, leading to adaptations upon mitochondrial impairment [20]. OG also signals deficits in nitrogen [21–24] on binding to either the transcription factor NtcA in cyanobacteria [22] or the phosphorylatable PII protein (*glnB* gene product) found in bacteria and plants [23,24]. Finally, OG acts as a repressor of the catabolite-sensitive genes in *E. coli*, partially through a decrease of cAMP synthesis [25]. The ensuing importance of the irreversible degradation of OG catalysed by OGDHC for general metabolic activity thus renders this multienzyme complex a promising target for metabolic regulation in a wide range of organisms. In the present review the enzymological properties of the complex are briefly summarized in order to show its catalytic and regulatory capacities for cellular disposal. Our main goal is to review recent data on the metabolic consequences of the OGDHC regulation in a diverse range of biological systems, such as cells, tissues and intact organisms.

CATALYTIC AND REGULATORY PROPERTIES OF OGDHC

OGDHC is a multienzyme system comprising the three catalytic components: OGDH (2-oxoglutarate dehydrogenase) (E1o, EC 1.2.4.2), dihydrolipoyl succinyl transferase (E2o, EC 2.3.1.61) and dihydrolipoyl dehydrogenase (E3, EC 1.8.1.4). Consecutive action of the components involves several cofactors [ThDP (thiamine diphosphate), Mg^{2+}, lipoic acid and FAD] and is described by reactions (1)–(5) in Scheme 1, resulting in OG oxidative decarboxylation according to the overall reaction (6), with R corresponding to $HOOC\text{-}CH_2CH_2$-.

The E1o component, which catalyses the initial, substrate-specific and irreversible stage of the overall reaction, has the lowest catalytic activity ('turnover number') among the components and as such limits the rate of the overall process (reaction 6) [26]. Within the E1o-catalysed process, reaction (2) has been demonstrated to be the slowest one [27,28]. The 'rate-limiting' role of E1o is obviously responsible for the fact that it is this component which is affected by the majority of the regulators of the overall process [16] and whose expression is often increased in response to the OGDHC impairments (see the section 'Genetic and proteomic evidence of the contribution of OGDHC to cellular networks' below).

OGDHC also catalyses several side reactions of potential biological significance [29–31]. These are the ROS (reactive oxygen species)-producing activity of OGDHC, which was shown to contribute to ROS generated by neurons in response to glutamate [32], and the carboligase reaction, which is supposed to participate in glyoxylate scavenging [33]. When the ratio of the OGDHC substrates and/or products promotes excessive formation of the dihydrolipate intermediate, OGDHC catalyses oxidation of the latter by molecular oxygen with the formation of the superoxide anion radical and thiyl radical of the complex-bound lipoate [29]. Figure 2 compares intensities of the superoxide-anion-radical production catalysed by OGDHC under different conditions as analysed by EPR detection of the adduct of the α-phenyl-*N*-t-butyl-nitron-trapped superoxide anion radical [29]. The reaction depends on the catalyst (OGDHC) concentration (Figure 2a), occurring either upon reduction of the complex-bound lipoate (Figures 2b and 2d) or in the presence of external dihydrolipoate (Figure 2c). The complex-bound lipoate is reduced in the direct reactions (1)–(3) (Figure 2b) or reversed reactions (5) and (4) (Figure 2d). The reaction kinetics presented in Figure 2



Scheme 1 Multistep process catalysed by OGDHC (R=HOOC-CH_2CH_2-)

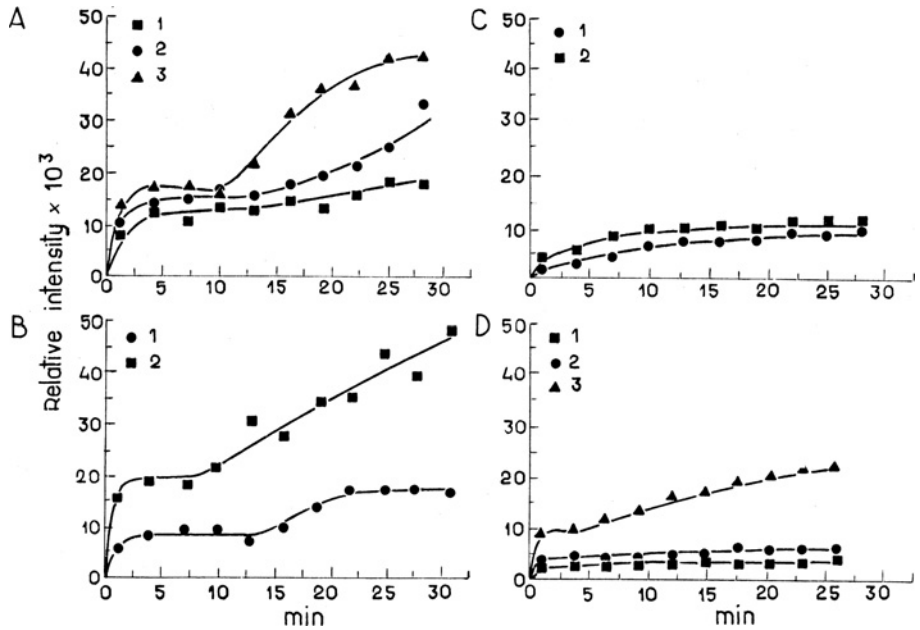


Figure 2 Enzyme- and substrate-dependence of the OGDHC-produced ROS

The EPR signals from the α-phenyl-*N*-t-butyl-nitron adducts with ROS produced in the medium with OGDHC and its substrates was detected as described in [29]. If not specified, 4 mg/ml enzyme and 4 mM substrate were used. (**A**) Enzyme: ■ 1, 1 mg/ml; ● 2, 2 mg/ml; and ▲ 3, 4 mg/ml. (**B**) OG and CoA: ● 1, 2 mM; ■ 2, 4 mM. (**C**) Dihydrolipoamide: ● 1, 1 mM; ■ 2, 2 mM. (D) NADH: ■ 1, 2.5 mM; ● 2, 5 mM; and ▲ 3, 17 mM.

are complex, owing to secondary processes, with the primary signal sensitive to superoxide dismutase [29]. Remarkably, the relative intensity of the adduct formation when the OGDHC is saturated with either OG and CoA (Figure 2b) or NADH (Figure 2c) indicates that ROS are more efficiently produced in the physiological direction of the reaction (Figure 2b) than upon oxidation of the product, NADH (Figure 2d). OGDHC-dependent production of ROS following supply of dihydrolipoate (Figure 2c), which is a substrate of both E2o and E3 components, provides a good explanation for the known pro-oxidant action of this dithiol, which is usually considered and employed as an antioxidant [34]. Importantly, the second product of the one-electron reduction of oxygen, the thyil radical of the complex-bound lipoate, inactivates E1o in the presence of OG, providing an expensive yet efficient regulation of OGDHC-dependent ROS production from OG [29,35].

The multienzyme structure of OGDHC is essential for regulation of both the physiological (reaction 6) and side reactions. With the 24 copies of E2o forming the OGDHC core, to which multiple copies of the peripheral E1o and E3 components are attached [36], OGDHC may be considered as a cellular microcompartment of lipoic acid. In this microcompartment not only is the overall reaction greatly accelerated and regulated [37], but also a number of side reactions of the multistep catalysis may be strictly controlled [15,35]. In particular, stability of the thiyl radical, afforded by the interactions of the lipoyl residues, facilitates efficient regulation of the ROS-producing activity of OGDHC according to the above-mentioned E1o inactivation [29], allowing OGDHC to respond to an integral signal of cellular metabolic state coded for by the ratio of its substrates and products [35]. Moreover, the thiyl-radical-dependent regulation also provides for the interplay between OGDHC and the thioredoxin system [35]. As a thiyl-radical scavenger [38], thioredoxin prevents E1o inactivation, facilitating the production of

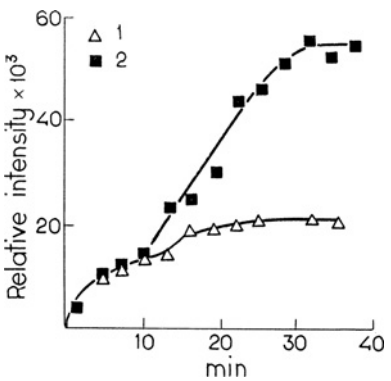
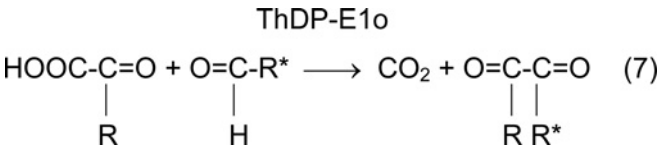


Figure 3 Effect of thioredoxin on the aerobic α-phenyl-*N*-t-butyl-nitron adducts produced by OGDHC (3.2 mg/ml) in the presence of OG and CoA (1.2 mM each)

The EPR signal was detected as described in [29]. Δ 1, Control without thioredoxin; ■ 2, in the presence of 2 mg/ml thioredoxin of *E. coli*.

succinyl-CoA under conditions in which the complex would normally be inactivated, i.e. at a low NAD^+ or when the $NADH/NAD^+$ ratio is high [39]. The biological significance of this regulation is supported by the independent observations that a most efficient regulation of mitochondrial OGDHC is provided by cognate mitochondrial thioredoxin [40], and the E2o component binds thioredoxin in high-throughput assays [41,42]. However, in protecting E1o from the inactivation by thiyl radicals, thioredoxin increases production of ROS by OGDHC (Figure 3). This fact may underlie an interesting observation that mammalian cells overexpressing mitochondrial (but not cytoplasmic) thioredoxin are characterized by an increased level of mitochondrial ROS [43].



Scheme 2 Carboligase reaction catalysed by E1

The carboligase reaction of OGDHC, catalysed by the ThDP-dependent E1o [30,31], is similar to reactions catalysed by non-oxidative thiamine enzymes, such as transketolase and acetolactate synthase. After OG decarboxylation according to reaction (1), the ThDP-bound intermediate is neither oxidized nor hydrolysed, but rather transferred to an acceptor substrate possessing a >C=O group. The process is described by reaction (7) (Scheme 2).

The efficiency of the reaction with glyoxylate (R*=-COOH) is obviously due to additional activation of the carbonyl carbon atom by the neighbouring carboxyl group. The biological significance of this process is confirmed by glyoxylate accumulation in tissues in which OGDHC is inhibited [33,44].

Since the regulatory mechanisms of the control of OGDHC by its multiple cofactors and substrates are considered in detail in another recent review [16], here we will only summarize them briefly. Regulation of the dimeric components E1o and E3 by their substrates and effectors include co-operative interactions of the active sites [45–49]. Besides, product inhibition of all the component enzymes is known, and allosteric regulation of E1o by the product of E3, NADH, has been demonstrated [49–53]. It should be also borne in mind that the multiple substrates and products of OGDHC (reaction 6), as well as the allosteric effectors not directly involved in reaction (6), such as adenine nucleotides and Ca^{2+}, exert their regulatory influence in a highly interactive manner, which makes the kinetic consequences of the regulation greatly dependent on conditions. Nevertheless, the product inhibition by NADH and succinyl-CoA, characterized *in vitro,* was shown to govern the OG oxidation in isolated mitochondria [50]. Most importantly, the multienzyme structure of OGDHC enables a concerted response from the component enzymes to regulate the physiological reaction according to a number of general indicators of cellular metabolism. As mentioned above, the microcompartment of the OGDHC-bound lipoate provides for regulatory interactions of the complex with cellular SH/-S-S-compounds and thioredoxin [35,39]. Increased ratios of NADH/NAD^+ and succinyl-CoA/CoA inhibit the OGDHC-catalysed process, whereas, in animal complexes, Ca^{2+} and ADP allosterically activate E1o at subsaturating concentrations of OG by increasing the E1o affinity to this substrate [45,47,52–54]. In plants, however, OGDHC responds to AMP instead of ADP [55, 56]. Unexpectedly, an allosteric AMP-binding site has been found in the crystal structure of bacterial E1o [57], although regulation of bacterial OGDHC by adenine nucleotides has not been reported. Neither was the calcium regulation of insect, plant or bacterial OGDHCs revealed, suggesting that this regulatory mechanism can distinguish vertebrate species [54].

DEVELOPING INHIBITORS OF OGDHC FOR *IN VIVO* STUDIES

Specific mechanism-based inhibitors with the phosphonate group substituting for the leaving carboxy group of OG

In 1977 and 1979, Kluger and Pike showed that the phosphono analogues of pyruvate, acetyl phosphonate and its methyl ester form transition-state analogue complexes with pyruvate dehydrogenase, causing efficient, but reversible, inhibition of the isolated enzyme [58,59]. Further studies of different thiamin-dependent enzymes transforming pyruvate, however, revealed that, in spite of the similarities of catalytic mechanisms, the efficiency of their inhibition by the phosphonate analogues of pyruvate is strongly variable [60]. Analysis of the phosphonate interaction with the enzymes pointed to the formation of the tight complexes, some of which have subsequently been crystallized [61,62], suggesting that these were strongly dependent on specific catalytic events. Bunik et al. [63] were the first to demonstrate potent inhibition of the animal OGDH component of OGDHC by the phosphonate analogue of OG, SP (succinyl phosphonate) (Figure 4), synthesized as described by Khomutov et al. [64]. Further studies indicated that the inhibition of the OGDH by this analogue, as well as some of its esters, was also extremely efficient within the native structure of the integral OGDHC from bacterial, plant and animal sources [63,65–68]. Three features of the mechanism of the OGDHC inhibition by the phosphonates established in these studies are worth mentioning regarding the efficiency and specificity of the action of these analogues *in vivo*. First, the phosphonates efficiently compete with OG at the active site of the OGDH component, with significant inhibition of brain OGDHC achieved at low (10^{-5} M) concentrations of the phosphonates under high saturation of the enzyme with OG (10^{-3} M). Given that, *in vivo*, OG concentrations range from 10^{-3} to 10^{-4} M [69,70], these data suggest a high inhibitory potential of the phosphonates for cellular studies. Secondly, the inhibition by the phosphonates occurs according to the slow tight-binding mechanism. That is, maximal inhibition requires a pre-incubation of the enzyme with the inhibitors. This is indicative of a kinetically slow conformational transition of the enzyme–inhibitor complex – a general property of the ternary complexes of the 2-oxo acid dehydrogenases with the coenzyme ThDP and phosphonate analogues of their respective substrates [58,59,63,68,71]. In these complexes the phosphonate analogues are bound much more strongly than in other enzymes, owing to formation of the transition-state analogues at the active sites. Unlike ordinary competitive inhibitors, transition-state analogues are known to not only bind much more tightly, but also to be formed much more specifically, which makes them especially attractive for targeting

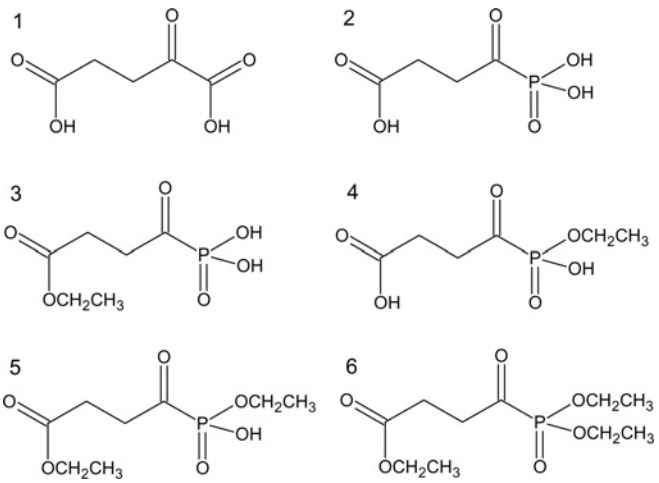


Figure 4 OG and its phosphonate analogues

1, OG; phosphonate analogues: 2, SP; 3, CESP; 4, PESP; 5, DESP; 6, TESP.

specific enzymes in cellular studies. Indeed, SP and its esters do not significantly interact with a number of other enzymes transforming OG and its structural analogues [66,67]. They are neither inhibitors [66,67], nor substrates of these enzymes, with the exception of a slow transamination of SP by transaminases [65,66]. The third general feature of the OGDHC inhibition by the phosphonate analogues of OG is that esterification of the phosphonate group of SP, both methylation [63,65] and ethylation [66,67,68,71], significantly decreases efficiency of the inhibition of both the OGDH component and full complex. This was observed for the enzymes from brain [68,71], muscle [63], *E. coli* [65] and a plant [67]. Preservation of the different efficiencies of the two analogues in their *in vivo* action may provide additional support for the OGDHC dependence of the *in vivo* effects of the phosphonates [71]. Remarkably, the degree of selectivity to SP and its monoethylated derivative was source-specific, reflecting certain structural differences in the species- and/or tissue-specific active sites, which potentially facilitates the species-specific drug design. In contrast with OGDH, pyruvate dehydrogenase reacts more readily with the methyl ester of the phosphonate analogue of pyruvate [58,59]. Therefore the mechanism of the inhibition of the 2-oxo acid dehydrogenases by the phosphonate analogues of their respective substrates provides for the high selectivity of the interaction, even within the same class of the ThDP-dependent enzymes. That is, phosphonate analogues of pyruvate do not affect OGDH, whereas phosphonate analogues of OG do not affect pyruvate dehydrogenase.

In line with their highly specific action on enzymes, the phosphono analogues of OG were shown to not significantly interfere with OG transport. OG (5 μM) uptake by proteoliposomes reconstituted with a mammalian peroxisomal OG transporter [72] was not inhibited by 1 mM SP, PESP (phosphonoethyl succinyl phosphonate) or DESP (diethyl succinyl phosphonate) (R.J. Wanders and W.F. Visser, personal communication). Mitochondrial transport was tested in proteoliposomes reconstituted with a recombinant bovine mitochondrial OG transporter ($K_m^{OG} = 0.3$ mM) as in [73]. The entry of external OG into proteoliposomes (i.e. transporter-catalysed OG exchange) was inhibited in the presence of 10 mM SP or PESP at 0.1 mM external OG ($K_m^{OG}/3$) by 18 and 24 % respectively. Increasing the external OG concentration to 3 mM decreased the inhibition to 7 or 14 % respectively, in agreement with the competitive inhibition expected. When the proteoliposomes were loaded in the presence of 10 mM OG, SP or PESP, and the entry of external OG (3 mM) into them was monitored over a period of 60 min, the SP- and PESP-loaded liposomes provided for only 2 and 3.7 % of OG entrance compared with the control value with the OG-loaded liposomes (F. Palmieri, personal communication). Thus the inhibition of the mitochondrial OG transporter by the phosphonate analogues of OG was insignificant, even at a 100-fold excess of the analogues on a 3-fold-less-than-K_m^{OG} concentration of OG (0.1 mM). That said, even a minor entry of OG in exchange with (PE)SP indicates an ability of the mitochondrial OG transporter to bring the phosphonates into mitochondria, although this process may also be dependent on the operation of additional transporters. It is also worth noting that, in both assays of OG transporter, PESP was more efficient than SP. In contrast, OGDHC is preferentially inhibited by SP [63,65–68,71]. This shows how comparison of the phosphonates with different selectivity to different target proteins may provide additional evidence for their targets *in vivo*. A higher *in vivo* effect of SP compared with PESP upon the glutamate neurotoxicity coincided with the OGDHC selectivity [71], which does not support the *in vivo* action of the phosphonates through the OG transporter.

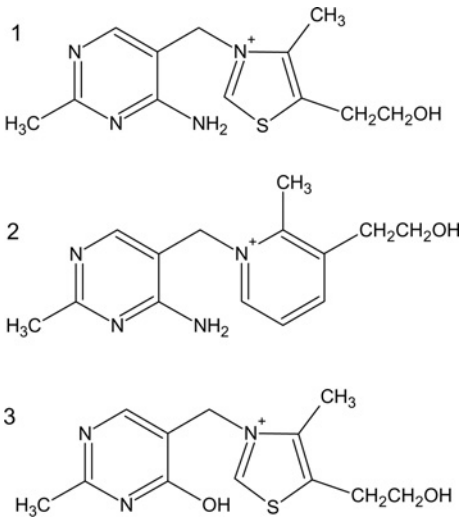


Figure 5 Thiamine (1) and its catalytically inactive analogues pyrithiamine (2) and oxythiamine (3)

Thus an inhibition mechanism as well as direct tests on enzymes and OG transporters provide strong evidence for the specificity of the inhibitors *in vivo*. Although these experiments cannot formally exclude other potential targets, comparison of different OGDHC inhibitors and independent studies discussed below further support the specific action of the phosphonate analogues of OG on OGDHC *in vivo*.

It should be borne in mind that the complete or partial esterification of the three negative charges of SP may increase the membrane permeability of the phosphonate analogues of OG. Inside a cell, these esterified derivatives are eventually transformed to SP by intracellular esterases, which is why the di- and tri-ethyl esters of SP, which do not inhibit OGDHC *in vitro*, cause inhibition of OGDHC in cells comparable with that caused by SP [32,66]. Thus the cellular application of the SP esters may be justified in cases when the membrane permeability of SP is supposed to be compromised. However, the SP effects observed in the non-permeabilized plant tissues [67] and neurons [32] provide evidence for its transport into native cells and mitochondria.

Thiamine antagonists as *in vivo* inhibitors of OGDHC

Catalytically inactive structural analogues of the OGDHC coenzyme ThDP (Figure 5) may also be used to decrease activity of the enzyme *in vivo*. Although such analogues should affect all thiamine-dependent enzymes, OGDHC was observed to be the physiologically relevant target both following pyrithiamine treatment [14,74–76] and when a single high dose of oxythiamine was injected [77]. By contrast, when the inhibition by the thiamine antagonists was studied *in vitro* using isolated thiamine-dependent enzymes of central metabolism, OGDHC was inhibited only by concentrations of the thiamine analogues that were three orders of magnitude higher than those affecting transketolase and pyruvate dehydrogenase [78]. This apparent discrepancy is resolved when taking into account different reactivities to the coenzyme analogues of the free (apo) and ThDP-bound (holo) enzymes. Unlike pyruvate dehydrogenase and transketolase, OGDHC isolated from normal animal tissues is a holoenzyme. That is, isolated animal OGDHC contains tightly bound ThDP

and does not require the coenzyme addition to the activity assay medium, whereas pyruvate dehydrogenase and transketolase do [78–80]. Hence, *in vitro*, this tightly bound ThDP at the active site of OGDHC obviously interferes with binding the ThDP analogue, which may, however, easily bind to the apoforms of pyruvate dehydrogenase and transketolase. In contrast, upon the thiamine deficiency *in vivo* the ThDP pool is exhausted, leading to a predominance of coenzyme-free (apo) forms of all thiamine enzymes. The preferential inhibition of apoform of OGDHC by the thiamine antagonists *in vivo* may therefore be due to the higher affinity of animal OGDHC for the coenzyme, potentially increasing the binding of catalytically inactive analogue as well.

The most widely used and best characterized approach to study thiamine deficiency employs pyrithiamine (Figure 5), which inhibits thiamine pyrophosphokinase, thus preventing thiamine phosphorylation [81]. As a consequence of this inhibition, pyrithiamine exhausts the pool of the coenzyme form of thiamine, ThDP. However, the compound was recently shown to act not only as an inhibitor, but also as a substrate for thiamine pyrophosphokinase, suggesting that production of the diphosphorylated form of pyrithiamine may occur *in vivo* [81]. The stronger binding of this analogue by the apoenzyme of E1o compared with other thiamine enzymes may explain the preferential targeting of OGDHC in the pyrithiamine model of thiamine deficiency, although the exact mechanisms underlying this specificity remain unresolved. Most importantly, the OGDHC activity levels and their responses to the treatment correlated with observed metabolic and behavioural changes, which established OGDHC as a physiologically relevant target of the pyrithiamine treatment in animals [75,76,82]. Hence a significant number of independent animal studies with pyrithiamine indicate that the results obtained with this inhibitor may be used for unravelling the physiological role of the OGDHC-catalysed process. Yet generally the thiamine analogues may target all the thiamine-dependent enzymes, with their specific target(s) under physiological conditions dependent on both the enzyme affinities for ThDP and analogues and saturation of the enzymes with endogenous ThDP. In this regard, the inhibition by the phosphonate analogues of 2-oxo acids is a much more direct and specific means to target the thiamine-dependent dehydrogenases *in vivo*.

OGDHC-DEPENDENT CONTROL OF MITOCHONDRIAL FUNCTIONS AND CELLULAR HOMOEOSTASIS IN ANIMALS

Respiration and mitochondrial potential

It has long been known that basic metabolic functions such as oxygen consumption correlate with the OGDHC activity per gram fresh weight in hearts under maximal physiological work load [83]. A strong correlation between the OGDHC activity and oxygen consumption was also observed upon OGDHC inhibition by arsenite in mitochondria isolated from cultured rat adrenal-medulla pheochromocytoma PC12 cells with elevated monoamine oxidase B [84]. Furthermore, brain mitochondria isolated from pyrithiamine-treated rats with inhibited OGDHC showed significantly depressed respiration on OG, but not on succinate [82]. Finally, using the specific inhibitors of OGDHC – SP and its triethyl ester, TESP (triethyl succinyl phosphonate) – the concentration-dependent decrease of the mitochondrial potential was observed upon glutamate stimulation in neuronal cultures [32]. All these data suggest significant control of the mitochondrial function by OGDHC, in particular under conditions of increasing energy demand, such as maximal heart work load, or neuronal stimulation by glutamate.

Cellular homoeostasis

Not only the respiration rate, but also its sensitivity to OGDHC inhibition, depend on the mitochondrial OGDHC activity. For instance, compared with cells overexpressing monoamine oxidase B, cells with normal levels of this enzyme displayed a 40 % higher OGDHC activity, which was accompanied by a 40 % 'spare OGDHC threshold capacity' – which is defined as the amount of enzyme which can be removed before compromising the rate of respiration [84]. This indicates that any deviation from the OGDHC activity level inherent in normal metabolism may restrict mitochondrial adaptability to changes in the intracellular milieu upon different types of cellular activities. This hypothesis is in good accordance with the documented association of a decrease in the brain OGDHC activity and neurodegenerative diseases [14], which was also observed in a cellular model of Parkinson's disease [84].

The role of OGDHC in glutamate-induced ROS production and excitotoxicity

In view of the *in vitro* studies showing that OGDHC inactivation occured with a certain ratio of the OGDHC substrates [29,35,39], it was suggested that metabolic disbalance under pathological states provokes the OGDHC inactivation [15]. Therefore the OGDHC protection from inactivation could be of potential neuroprotective value. *In vitro*, the catalysis-associated inactivation could be alleviated, in particular, by the binding of the OG structural analogues to the first component of the complex, OGDH [85]. The synthetic OG analogue SP protected OGDHC from the inactivation at several orders of magnitude lower concentrations than the natural analogue malonate [68]. This protective effect on OGDHC of relatively low (10^{-4} M) concentrations of SP is in line with the neuroprotective action of SP in a model of neurodegeneration induced by the glutamate overstimulation of cultivated neurons [68,71]. Our analysis of available biochemical data [68] showed that overstimulation with glutamate should increase the OGDHC-dependent side reactions, i.e. production of ROS and associated enzyme inactivation. Indeed, neuronal ROS production is known to increase upon overstimulation with glutamate [86]. The OGDHC contribution to this process was demonstrated in the physiologically relevant mixed culture of neurons and astrocytes through the inhibition of the glutamate-induced neuronal ROS by the phosphonate analogues of OG [32]. Simultaneously with the reduction in ROS, the neurons loaded with SP became more resistant to other disturbances in cellular homoeostasis caused by glutamate, such as delayed Ca^{2+} deregulation and irreversible mitochondrial depolarization [68,71]. Thus application of specific effectors of OGDHC showed the critical role of OGDHC in the long-known association between the glutamate overstimulation and mitochondrial impairment [87], explaining this association at the molecular level. That is, the OGDHC inhibition by phosphonates under conditions of the glutamate overstimulation decreases the OGDHC-dependent neuronal ROS production induced by glutamate. Concomitantly, the E1o inactivation by the thiyl radicals (see the section 'Catalytic and regulatory properties of OGDHC' above) is alleviated. As a result, propagation of the vicious cycle of metabolic disbalance arising secondary to the OGDHC inactivation under increased glutamate supply to the tricarboxylic acid cycle, is blocked. Thus application of the phosphonate analogues of OG facilitates the maintenance of neuronal homoeostasis upon glutamate overstimulation.

Metabolic shifts following the *in situ* and *in vivo* inhibition of OGDHC

In pyrithiamine-treated animals, a decrease in the brain OGDHC activity was associated with an increase in the extracellular glutamate within the vulnerable brain regions, suggesting that glutamate excitotoxicity causes neuronal damage in these regions [74,88,89]. A recent study revealed a strong correlation between regional brain vulnerability in this model and early metabolic changes, establishing decreases in aspartate levels as a predictor for vulnerability [75]. These data are complementary to the expected metabolic consequences of the OGDHC inhibition and to perturbations in amino acid metabolism observed in neuronal culture treated with PESP and CESP [90]. A good coincidence in metabolic consequences of the OGDHC inhibition by different means (see also sections 'Correlation of OGDHC function and physiology in intact animals' and 'Genetic and proteomic evidence of the contribution of OGDHC to cellular networks' below) further supports specificity of the action of OGDHC inhibitors *in vivo*, as discussed in the section 'Developing inhibitors of OGDHC for *in vivo* studies' above. In the latter study, OGDHC inhibition in neurons was accompanied by an up-to-2-fold decrease in aspartate (Figure 6A). Several other amino acids, most consistently GABA (γ-aminobutyric acid) and alanine, showed a decrease in all these studies too [74,90] (Figure 6A), probably a result of their transamination with the OG that accumulated on OGDHC inhibition. The level of intraneuronal glutamate, however, did not show a statistically significant change under these conditions [90], it being decreased rather than increased (Figure 6A). When considered alongside the increased extracellular glutamate observed in independent studies [88,89], no increase in glutamate inside neurons [90] suggests an elevated excretion of glutamate from the neurons upon OGDHC inhibition. This is consistent with the observed decrease in the intraneuronal pool of [4-^{13}C]glutamate synthesized from [1-^{13}C]glucose, which was also documented following phosphonate inhibition of OGDHC [90].

Conditional consequences of OGDHC inhibition

It should be noted that significant inhibition of OGDHC, which induced the above-mentioned metabolic shifts in neuronal culture, required two-orders-of-magnitude-higher concentrations (10^{-2} M) of the phosphonate analogues [90] compared with those used in the neuroprotective studies (10^{-4} M) [68,71,91]. Obviously the metabolic consequences of the OGDHC inhibition may be different depending on the degree of inhibition as well as the conditions under which the inhibitors are applied. Glutamate excitotoxicity has been suggested to lead to an increased flux through OGDHC [68] as a consequence of calcium activation of both OGDHC [45] and glutamate import into the mitochondria [92]. Considering these phenomena, glutamate oxidation in the tricarboxylic acid cycle should be increased upon the incubation of neurons with glutamate, leading to their overstimulation. Mitochondrial hyperpolarization following provision of glutamate to neurons supports this hypothesis [86,93]. Under these conditions the inhibitory action of the analogues corrects the flux perturbation and metabolic imbalance by inhibiting not only the excessive NADH production by OGDHC, but also its side reactions, such as the production of ROS and reactive intermediates that irreversibly inactivate E1o [35,68,85]. Thus low concentrations of phosphonates on the glutamate overstimulation provide a neuroprotective effect [68,71], whereas high concentrations of phosphonates inhibiting normal metabolism [90] may model the situation of the OGDHC activity decrease associated with neurodegeneration [14,15]. Such biphasicity is often observed with agents affecting signalling and viability of complex biological systems. For instance, hydrogen peroxide acting through the p53 transcription factor elicits antioxidant gene expression at low concentrations, but pro-oxidant genes are expressed at high concentrations of hydrogen peroxide [94].

Correlation of OGDHC function and physiology in intact animals

Pyrithiamine-treated rats were characterized by a decrease in OGDHC activity which was restored concomitantly with metabolic and behavioural changes following thiamine restoration [74,76,95,96]. The motor deficiency of the pyrithiamine-treated animals correlated with the OGDHC impairment in the most vulnerable brain region – the submedial thalamic nucleus [97]. A decrease in aspartate in specific brain regions of the pyrithiamine-treated rats was demonstrated to be an early marker of regional vulnerability of the brain to the treatment [75]. Attribution of this decrease to OGDHC inhibition was confirmed by an independent study where a significant, up to 2-fold, decrease in neuronal aspartate levels was observed as a result of the OGDHC inhibition by the phosphonates (Figure 6A; [90]). OGDHC inhibition is involved also in another hallmark of thiamine deficiency, namely the accumulation of glyoxylate and its derivative oxalate [44]. As discussed in the section 'Catalytic and regulatory properties of OGDHC' above, the E1o component of OGDHC may scavenge glyoxylate by catalysing the ThDP-dependent carboligase reaction between OG and glyoxylate [30,31]. Inhibition of this reaction in the pyrithiamine-treated animals was shown [33], suggesting its contribution to glyoxylate-induced damage, such as inactivation of several tricarboxylic-acid-cycle enzymes, including OGDHC [98–100]. Thus independent studies of different metabolic impairments in the pyrithiamine-treated rats document a leading role for OGDHC inactivation in the physiological consequences of pyrithiamine-induced thiamine deficiency.

The action of SP in animals has revealed the developmental impact of OGDHC activity [91]. That is, an intranasal supply of SP to pregnant rats leads to changes in biochemical, morphometric and physiological parameters in their offspring. Female offspring were characterized by increases in the total activity of OGDHC in cerebellum and cortex concomitantly with increasing the mass of these brain structures. Furthermore, both male and female offspring displayed altered electrocardiograms. Remarkably, the developmental changes induced by SP are similar to those induced by hypoxic treatment of pregnant rats [91]. This is in good accordance with the inhibition of the respiration expected under both conditions, given that OGDHC exerts significant control over this process (see the section 'Respiration and mitochondrial potential' above). Moreover, like SP inhibition (see the section 'Metabolic shifts following the *in situ* and *in vivo* inhibition of OGDHC' above and Figure 6a), hypoxia is known to change the levels of amino acid neurotransmitters such as glutamate, GABA and aspartate [101–103]. Most interestingly, introduction of SP before the onset of hypoxia revealed an antagonistic relationship between the two insults, abolishing the changes observed upon independent application of either factors. Protection from hypoxia-induced changes, provided by the application of SP, may well be due to its neuroprotective action upon glutamate excitotoxicity [68,71,91], as the latter process is known to greatly contribute to hypoxic damage [101–103].

The developmental changes following application of the OGDHC inhibitor SP [91] are in good accordance with mutational studies, which revealed that non-functional OGDHC causes

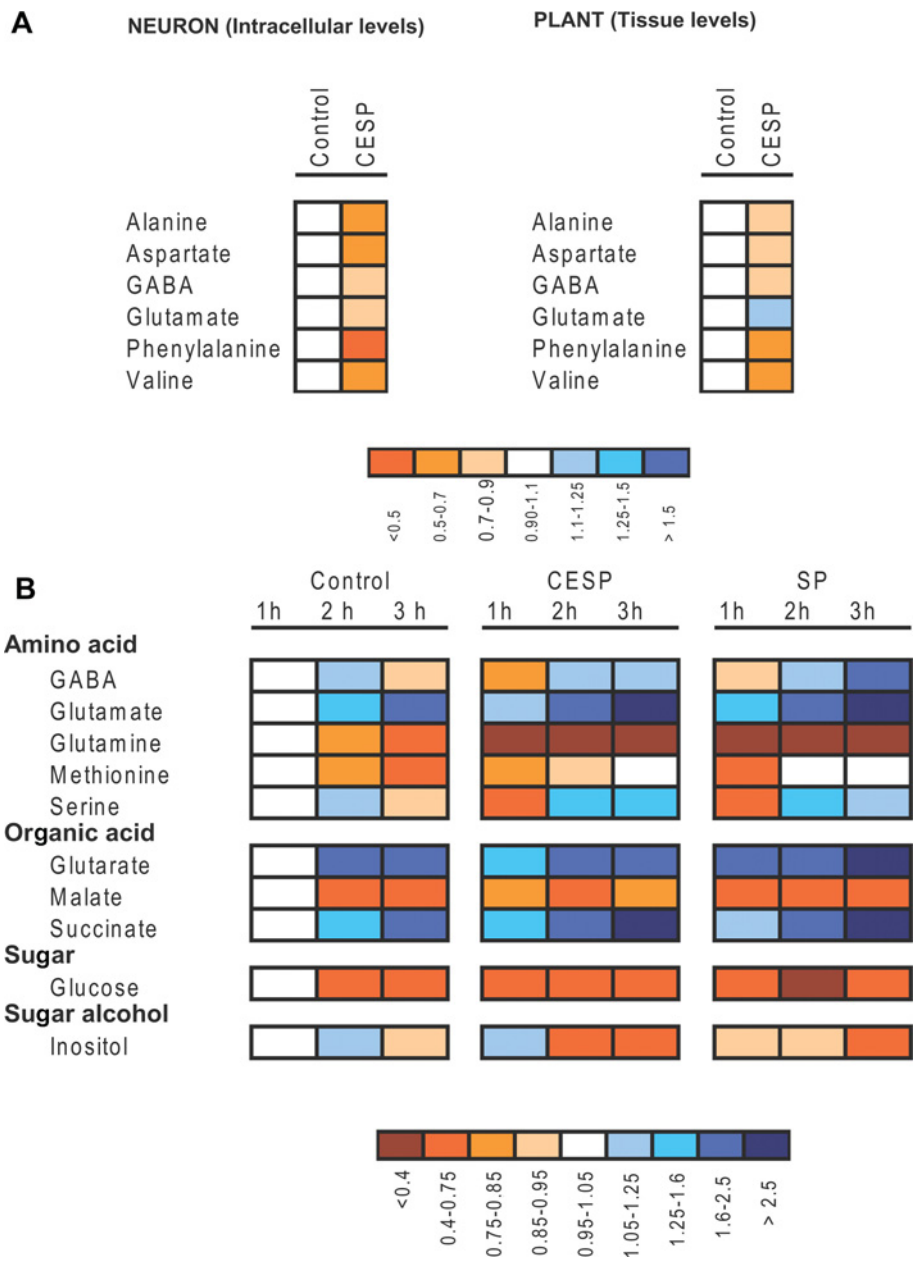


Figure 6 Heat maps representing the changes in relative metabolite levels of phosphonate-treated samples

(**A**) Heat map comparing response of plant and neuronal tissue to application of CESP; all metabolites common to both data sets are displayed (data from [67] and [90]). In both cases data were normalized with respect to the control mean. (**B**) Heat map showing the ten metabolites that were strongly correlated with changes in OG levels following application of phosphonate inhibitors to intact potato tubers (data from [67]).

death during early stages of embryonic development [7,9]. Moreover, even less severe impairments in OGDHC cannot be tolerated, resulting in death before adulthood [10–13]. In view of the OGDHC control of brain glutamate metabolism, it seems reasonable to speculate that it is this control which provides for the intimate relationship between OGDHC activity and development of the central nervous system, where glutamate is known to regulate proliferation, migration and survival of both neuronal progenitors and immature neurons [104,105]. This is further supported by the observation that defects in OGDHC are accompanied by a decreased number of neural progenitor cells in adult mice [106].

OGDHC-DEPENDENT CONTROL OF MITOCHONDRIAL FUNCTIONS AND CELLULAR HOMOEOSTASIS IN PLANTS

Plant OGDHC plays an important role in the regulation of respiration

Incubation of potato (*Solanum tuberosum*) tuber discs, which form a useful model system for studying plant heterotrophic metabolism (see [107] for an explanation), was recently performed, in the presence and absence of the phosphonate inhibitors SP or CESP, in order to evaluate the importance of OGDHC in respiration [67]. The presence of either inhibitor markedly decreased the rate of respiration over the entire time

course (4 h) of the experiment. However, inhibition was less strong following 3 h of incubation (~30 %) than following 2 h of incubation (~50 %). As a result, the respiratory burst, known to be imposed by wounding due to the tuber disc preparation [107,108], was dramatically slowed down by the OGDHC inhibitors. Given that another typical response to wounding is increased ROS production [109,110], it seems reasonable to speculate that the respiratory burst upon wounding is, at least in part, to meet the ROS production in wounded tissues. In this regard, our data on the respiratory burst inhibition by phosphonates suggest an OGDHC contribution to the stress-induced ROS in plant tissues similar to that observed in neurons (see the section 'The role of OGDHC in glutamate-induced ROS production and excitotoxicity' above).

Intriguingly the decreases in respiration following phosphonate inhibition of OGDHC [67] were far in excess of those previously reported following the transgenic inhibition of mitochondrial isoforms of malate dehydrogenase [111], fumarase [112], citrate synthase [113] and succinyl-CoA ligase [114] or the decreased activities of the NAD^+-dependent isocitrate dehydrogenase [115] or aconitase [116] in mutants of *Arabidopsis thaliana* (thale cress) and tomato [*Solanum lycopersicum* L. (formerly *Lycopersicon esculentum* Miller)] respectively. This comparison thus affords the important conclusion that the OGDHC reaction is of prime importance in the control of respiration in comparison with other enzymes of the tricarboxylic acid cycle.

Further experiments evaluated the rate of $^{14}CO_2$ evolution following incubation of tuber discs in [1-^{14}C]OG or [1,2-^{14}C]acetate in the presence and absence of SP and CESP. The labelled substrates are respectively the direct precursor and an often used surrogate precursor for tricarboxylic-acid-cycle activity in general [67,117,118]. Although inhibitors caused a decrease in $^{14}CO_2$ evolution following incubation in both [1-^{14}C]OG and [1,2-^{14}C]acetate, at later stages of incubation the degree of inhibition was much higher in those samples incubated in [1,2-^{14}C]acetate. This could be due to differences in the labelling distribution of the substrates. Alternatively, it could result from the isocitrate dehydrogenase inhibition induced by metabolic changes upon the OGDHC inhibition. Direct inhibition of the isocitrate dehydrogenase by the phosphonates was not shown to occur [67].

Involvement of plant OGDHC in nitrate assimilation

The majority of attention regarding nitrate assimilation has been afforded to the reaction step catalysed by isocitrate dehydrogenase (for a review, see [23]). However, the reactions catalysed by citrate synthase [113] and OGDHC have recently been considered [67]. Phosphonate inhibition of potato tuber OGDHC causes a clear inhibition in nitrate assimilation, as the tissue concentration of NO_3^- was elevated in the inhibitor-treated samples [67]. Application of a broad GC–MS-based metabolite profiling method [119,120] allowed unprecedented characterization of the metabolic consequences of phosphonate inhibition (for details, see [67]). Among the metabolic changes observed were the increases of the levels of several amino acids relative to that found in control incubations, including glutamate and GABA, which were likely to be due to increased transamination of accumulated OG. These increases in amino acid content in isolated tuber discs are consistent with the recent discovery that potato tuber has the entire complement of enzymic machinery for *de novo* amino acid biosynthesis [121,122]. Perhaps more importantly, however, elevated content of these key indicators of nitrogen status may be responsible for inhibition of the nitrogen assimilation, providing a feasible explanation for elevated tissue NO_3^-.

Although multiple changes in metabolites were reported following inhibition of OGDHC, metabolites whose levels were significantly correlated with OG across the experiment (Figure 6B) further support a role for OGDHC in nitrate assimilation. In accordance with this role, the amino acids glutamate, glutamine and GABA were among the ten metabolites which displayed a tight association with the cellular level of OG (positive in the case of glutamate and GABA, but negative in the case of glutamine; Figure 6B). This finding clearly implicates the importance of OGDHC for the juncture of the tricarboxylic acid cycle and nitrogen assimilation, adding to the longheld view that isocitrate dehydrogenase is the major regulatory step at the juncture [123–125]. When considered together, these studies thus suggest that activities of both enzymes, and by implication the mitochondrial transporter responsible for the transport of OG [126], play a crucial role in nitrate assimilation. Importantly, comparison of these data with those obtained in a less extensive study in neuronal cultures revealed clear, cross-kingdom, commonality in the sensitivity of the levels of amino acids to the treatment with the phosphonate analogues (Figure 6A). Although a particular amino acid may either increase or decrease, which obviously depends on the type of assay (the whole tissue or inside the cells only) and the inhibition level (defined by the phosphonate concentration and/or time of treatment), the changes in the amino acid pools (Figure 6) clearly highlight the conserved importance of OGDHC for the juncture of the tricarboxylic acid cycle and nitrogen assimilation.

Involvement of plant OGDHC in other metabolic processes

Along with increases in the tissue levels of nitrate, glutamate, glutamine and OG, Araujo et al. [67] also documented significant positive correlations between the levels of OG and glutarate, methionine and succinate. By contrast, glucose, inositol, serine and malate showed a negative correlation with the OG levels. Furthermore, non-correlative increases in the levels of alanine were suggestive of a stimulation of fermentation. In order to substantiate the finding of a broader influence of OGDHC on primary metabolism, a range of isotope-labelling experiments were carried out. In the simplest of these, [U-^{14}C] glucose was fed to potato-tuber discs incubated in the presence or absence of the phosphonates [67]. This experiment revealed an overall depression of metabolism, as evidenced by the marked decrease in radiolabel uptake, as well as an especially high decrease in label redistribution to CO_2 and the cell-wall fraction. Interestingly, label redistribution to starch and sucrose showed complementary biphasic changes. That is, labelling in starch and sucrose was increased and decreased respectively at low concentrations of both SP and CESP, returning to the control levels upon increasing the inhibitors [67]. This phenomenon mimics the biphasic effects of OGDHC inhibition reported in animal systems (see the sections 'Conditional consequences of OGDHC inhibition' above and 'Physiological conditions inducing changes in the synthesis of the OGDHC components' below).

Two further experiments were carried out, namely the feeding of [^{13}C]pyruvate and [^{13}C]glutamate, under the same conditions, in order to assess whether compensatory changes in flux through fermentation or the GABA shunt, do indeed occur following treatment with the inhibitors. In both instances this was shown to be the case, with particularly large increases in the fermentative flux. However, changes in the GABA shunt suggest that its up-regulation helps to maintain succinate supply to the mitochondrial electron-transport chain. This finding, when taken together with those from previous studies, highlights the metabolic importance of the GABA shunt in plants [127] and is similar to findings observed following neuronal OGDHC inhibition [90].

GENETIC AND PROTEOMIC EVIDENCE OF THE CONTRIBUTION OF OGDHC TO CELLULAR NETWORKS

Genetic impairment of the synthesis of OGDHC components has been studied in both bacteria [128,129] and yeast [133–135]. However, in higher eukaryotes, few genetic studies have been documented to date. In animals, this is mainly due to the fact that the null mutants of the OGDHC components are non-viable [7,9–13] Remarkably, in bacteria, a low constitutive expression of the E1o gene was also observed under all conditions, including anaerobiosis [133], and disruption of the E1o gene adversely affected survival in stationary phase [128]. As yet no reports exists concerning the genetic manipulation of the enzyme in plants. However, for the sake of comprehensivness, we document below some of our own preliminary observations of the consequences that deficiency of its expression confer in these species.

Bacterial OGDHC

The expression of the OGDHC components in *E. coli* has been demonstrated to be strongly dependent on the availability of oxygen and on carbon source, being regulated by ArcA(B) (aerobic respiratory control 2-component system) and FNR (ferric nitrite reductase) transcription factors [133–135]. In particular, the expression of the E1o-coding *sucA* gene from the upstream sdhC promoter varied by up to 14-fold depending on the carbon substrate used, and displayed a 4-fold increase under aerobic conditions [133]. Expression of the E3 component also increased 5-fold upon aerobic growth, with 11- and 15-fold increases in the E3 expression observed respectively in the FNR and ArcAB deletion strains [134]. Although the terminal E3 component is common to all 2-oxo acid dehydrogenase complexes as well as the glycine decarboxylase system, bacterial OGDHC is known to bind E3 approx. 30-fold less efficiently than the pyruvate dehydrogenase complex [136]. Since the isolated complexes usually show a significant degree of saturation with the E3 component [51,136], a change in E3 expression would be expected to preferentially alter the activity of OGDHC above that of the pyruvate dehydrogenase complex.

The absence of a functional OGDHC changes the carbon-source-dependence of bacteria [129] and is usually considered in view of the biosynthetic/assimilatory role of the initial module of the tricarboxylic acid cycle which leads to accumulation of OG and glutamate [21,22,137–139]. In the facultative methylotroph *Pseudomonas* AM1, inactivation of E2o via mutation results in obligate methylotrophy, i.e. the mutant can only grow normally on C_1 compounds [129]. Interestingly, proteomic studies revealed that carbon limitation in *E. coli* leads to oxidative modification of several tricarboxylic-acid-cycle (E2o, aconitase, succinyl-CoA ligase, and malate dehydrogenases) and associated (pyruvate dehydrogenase) enzymes [140]. These changes may be employed, alongside the transcriptional changes described above, as an additional mechanism of switching off the tricarboxylic acid cycle via protein inactivation by post-translational oxidation.

The importance of the regulation of OGDHC in bacterial glutamate synthesis is underlined by the existence of a specific regulatory protein capable of increasing glutamate production in corynebacteria. Highly efficient binding of this protein to OGDHC inhibits oxidative decaboxylation of OG, with the strength of this interaction controlled through the inhibitor phosphorylation by the protein kinase PknG [139]. In view of these data, one may speculate that the recently discovered thiamine derivatives synthesized in *E. coli* in response to carbon or amino acid starvation, such as adenosine thiamine triphosphate or thiamine triphosphate [141,142], may act as alarmones by targeting OGDHC. Identification of an allosteric AMP-binding site in the ThDP-binding E1o of *E. coli* [57] supports this speculation by providing a structural basis for regulatory binding of the ligand (adenosine thiamine triphosphate) which comprises cross-linked ThDP and AMP moieties.

Although the lack of detectable OGDHC activity in a number of micro-organisms and fungi has often been considered as an evidence for their lacking a functional complex, previously obtained genome sequences and improved activity assays have indicated that many of these species do indeed possess OGDHC [128,143]. Moreover, disruption of *sucA* in *Nitrosomonas europaea*, an autotrophic nitrifying bacterium which lacks measurable OGDHC activity, but possesses all the genes for synthesis of its component enzymes, affected survival upon changing conditions of stationary growth [128]. As mentioned above, a constitutive expression of the OGDHC components was also observed under anaerobic conditions in *E. coli* [133]. It is thus possible that OGDHC may participate in cellular network regulation under conditions which do not require OGDHC-dependent energy production. For instance, OGDHC-dependent ROS and thiyl-radical generation regulated by thioredoxin (see the section 'Catalytic and regulatory properties of OGDHC' above) may well contribute to cellular signalling and redox-sensitive regulation of metabolism [35]. In particular, this function of OGDHC could explain the hitherto unclear connection between the E1o component and the presumably redox-sensitive ammonia mono-oxygenase in *Nitrosomonas* [128].

Yeast OGDHC

Transcription of the OGDHC component genes is subject to common regulators in yeast (see [131] and references cited therein). The transcription regulatory proteins HAP 2 and HAP 3 activate the transcription of the genes for E1o, E2o and E3. In addition, the transcription is catabolite-repressed, with the higher levels of the mRNAs detected upon yeast growth on the non-repressible sugar galactose than on glucose. Mutants in the genes encoding the E1o and E2o components of yeast are respiration-deficient [130,131]. They can grow on minimal medium containing glucose, but not on glycerol, as the sole carbon source. The same growth phenotype was inherent in still another yeast mutant of a nuclear gene most probably controlling the binding of E1o to the E2o core. This mutant was deficient in OGDHC activity owing to impaired assembly of the complex, although the level of the component expression and E2o lipoylation were normal [132]. The presence of a functional pyruvate dehydrogenase complex in the mutant strains excluded the possibility that the mutation might affect proteins or cofactors shared between the two complexes such as dihydrolipoamide dehydrogenase, lipoic acid, thiamine pyrophosphate and FAD. These observations thus suggest that a special protein is required for the correct integration of E1o into the complex in yeast.

Genetic manipulation was utilized to demonstrate that assembly of yeast OGDHC with native stoichiometry between the components requires a balanced expression levels of the first and second components. That is, overexpression of E2o relative to E1o results in a preponderance of incompletely assembled complexes with substoichiometric amounts of E1o [132].

Animal OGDHC

Regulation of the OGDHC component expression

In animals, the genes coding for the OGDHC components, all nuclear, are not neighbouring, even when belonging to one

chromosome (e.g., human E1o and E3 components). More often they are found on different chromosomes, and their co-regulated expression pattern is unknown. Regarding the first component of OGDHC, E1o, only the regulation of the gene on the seventh human chromosome, encoding the well-known (heart) isoform of the OGDH, has been studied. Glutamate increases the OG-induced up-regulation of the promoter activity of this gene, but is inactive alone. ThDP, thiamine and its analogues, dibutyryl cAMP, dexamethasone, lactate and tricarboxylic-acid-cycle intermediates, except OG, showed no effect on the transcription [144]. The brain isoform of E1o (tenth human chromosome) has only recently been discovered [145] and its gene regulation pattern is unknown. Human E2o gene (fourteenth chromosome) contains regulatory sequences resembling glucocorticoid-responsive and cAMP-responsive elements, and a binding site for a cellular transcription factor, Sp1 [146]. The human E3 gene (seventh chromosome) was also shown to possess the cAMP-response element-like site. Interestingly, with basal transcription of the human E3 promoter essentially dependent on CREB (cAMP-response-element binding protein), no cAMP-dependent gene regulation was revealed [147].

Physiological consequences of the decreased synthesis of the OGDHC components in animals

As noted above, null mutants of the E2o and E3 components of OGDHC lead to non-viable organisms. By day 5 of embryonic development, the phenotype of the zebrafish E2o-null mutant was characterized by a narrow pointy head, slightly smaller eyes, a ventrally jutting jaw and a slightly underdeveloped gut [7]. The defects in the head suggest an impaired development of ectoderm-generated nervous tissue, in line with previous findings that associated neurological symptoms with genetic deficiencies in OGDHC activity [10–13]. In E3-null mutant mice the situation is considerably more complex, since all 2-oxo acid dehydrogenase complexes are affected. These organisms die prenatally, with the embryos ceasing to develop shortly after implantation, pointing to the requirement of E3 during the perigastrulation period [9]. Despite the fact that E3 is included in all of the 2-oxo acid dehydrogenase complexes, an E3 mutation in humans was identified that specifically impairs OGDHC activity [12,13]. This is obviously the consequence of the known differences in the binding sites of E3 in the different complexes. In particular, mammalian OGDH binds E3 through the E1o component [148], whereas the pyruvate dehydrogenase complex includes a specific E3-binding protein, albeit one that is structurally homologous with E2p [149]. As discussed above, the differences in the affinity of different complexes to the common E3 component [136] may also contribute to the complex-specific regulation by the E3 expression. Moreover, the brain-specific expression of the E1o isoform correlates with the decreased amount of the E3 component in the brain OGDHC, which points to the possibility of tissue-specific regulation of the E3 expression and binding to OGDHC [145].

Heterozygous mutant mice exhibiting genetic lesions in E2o or E3 [106,150] displayed increased lipid peroxidation, which was accompanied with a specific decrease in the number of immature neurons in the hippocampal dentate gyrus, but not in the subventricular neurogenic zone. The mutants also exhibited less resistance to lesions induced by mitochondrial toxins [150]. These data must be interpreted with caution, since one may expect multiple pleiotropic changes in the heterozygous mutants. Besides, the properties of the E3 knockout cannot be specifically attributed to OGDHC, since the E3 component is common to the pyruvate, OG and branched chain 2-oxo acid dehydrogenase complexes, as well as to the glycine decarboxylase system. However, that study shows that the mitochondrial defects, including those in OGDHC (E2o mutant) can reduce adult neurogenesis.

Cellular levels of E2o were also decreased in human embryonic cells via transfection with an antisense expression vector [151]. This transformation reduced cellular growth under standard conditions and increased both ROS levels and cell death following the application of hydrogen peroxide. Remarkably, a severe ($\sim$70 %) decrease in E2o induced a compensatory increase ($\sim$40 %) in the cellular synthesis of the E1o component. However, despite restoration of the OGDHC activity, cellular competence was not fully recovered, suggesting that the perturbed ratio of the complex components negatively affects cellular functions. The increased concentration of the non-bound E1o could be damaging, either in itself or owing to the stimulation of E1o-catalysed side reactions [15]. For instance, accumulation of extramitochondrial E2o due to its impaired degradation may give rise to anti-E2o antibodies produced in primary biliary cirrhosis [152]. In addition, the OGDHC-transmitted signalling through the substrate- and thioredoxin-dependent ROS generation [35] could be perturbed. Finally, mammalian E2o could conceivably be involved in several functions beyond those fulfilled in OGDHC. In keeping with this hypothesis, a truncated E2o, localized to the intermembrane space of mitochondria, appears to be involved in the biogenesis of the brain mitochondrial respiratory complexes [153], and a polypeptide derived from E2o was found in the plasma membrane of skeletal muscle [154]. The existence of the extramitochondrial E2o provides a rationale for an ubiquitin-dependent pathway of E2o degradation in the cytoplasm [152].

Physiological conditions inducing changes in the synthesis of the OGDHC components

Comparative analysis of the protein and/or its mRNA level may reveal metabolic conditions under which expression of the OGDHC components is altered, providing hints for the role of OGDHC in cellular adaptation. A 3-fold increase in the protein content of the E1o component of OGDHC was observed in liver mitochondria of rats exposed to ethanol [155]. Given that chronic alcohol consumption in rats is known to decrease OGDHC activity [156], the observation of increased protein levels of the rate-limiting component E1o [155] may well represent a compensatory response of a cell to the overall decrease in OGDHC activity. The same type of cellular compensatory response was documented under circumstances in which cellular E2o synthesis was decreased [151]. Further evidence for this compensation was provided by reverse-transcription–PCR assays, which were performed in order to define the spatiotemporal specificity of OGDHC component expression in response to pyrithiamine treatment in the rat model of thiamine deficiency [97]. A significant decrease in OGDHC activity at a late stage of treatment was accompanied by a decrease in the mRNAs of all components of the complex. Such a down-regulation in protein synthesis could conceivably be envisaged as a result of an energy deficit, especially because the decrease in both the OGDHC activity and mRNA levels was substantially less in non-vulnerable (cortex) than vulnerable (submedial thalamic nucleus) regions. However, prior to the abovementioned pronounced decreases in mRNA levels and protein activities, the cortex, in contrast with the submedial thalamic nucleus, displayed a 25 % increase in the E1o component mRNA [97]. On the basis of these results we suggest that there is a more efficient compensatory ability in the

cortex as opposed to the submedial thalamic nucleus, which may underlie a greater resistance of the cortex to OGDHC inhibition.

The abundance of the E2o component of OGDHC was increased 2-fold in hearts of exercise-trained rats [157], a finding in good accordance with the expected increase of general physiological capacity afforded by such a challenge. In the brains of pyrithiamine-treated animals, mRNA for the E2o component revealed no statistically significant changes in the non-vulnerable cortex, but was decreased in the vulnerable submedial thalamic nucleus.

Potassium deficiency has been reported to lead to a 2-fold increase in E3 levels in skeletal muscle [158]. Similar to the situations documented above, such an increase may represent a compensatory response aimed at ameliorating the decreased OGDHC activity, since potassium is known to stabilize the binding of ThDP to E1o [80]. That said, potassium performs a similar function in E1p [159] and, as noted above, increased expression of the terminal E3 component, which is common to all of the 2-oxo acid dehydrogenase complexes as well as the glycine decarboxylase complex, may affect the four systems. Interestingly, during pyrithiamine treatment, E3 mRNA in the non-vulnerable cortex was initially decreased and then increased, whereas in the vulnerable submedial thalamus the opposite changes in the E3 mRNA abundance were detected [97]. Thus, although E3 does not limit the rate of the overall reaction under saturating substrates *in vitro* [26], its expression is subject to regulation under conditions potentially decreasing the *in vivo* OGDHC activity. In this regard it must be stressed that cellular adaptations by regulation of E3 synthesis might be due to regulation of not only the NADH production, but also the associated side reactions of E3 within the 2-oxo acid dehydrogenase complexes [15,35]. In particular, a diminished E3 content of the complex may decrease the ROS-producing activity of OGDHC [29]. So far, decreased expression of E3 observed at an initial stage of the pyrithiamine treatment in non-vulnerable cortex, but not in vulnerable submedial thalamus (see above), may be providing for cellular resistance to metabolic imbalance concomitant with the increased expression of E1o component.

Plant OGDHC

Surprisingly, given the fact that a multitude of studies in *Arabidopsis*, tomato and tobacco have been reported for other enzymes of the tricarboxylic acid cycle (see [112,160,161] for an overview), as yet there are no reports of studies employing mutagenic or transgenic approaches to genetically manipulate the activity of OGDHC. That said, surveying insertional populations of the model plant *A. thaliana* (http://www.arabidopsis.org/portals/mutants/worldwide.jsp) revealed the presence of a number of mutants in all subunits. Although detailed molecular characterization of the majority of these has not yet been carried out, we can state that null mutants of the E1o subunit of *Arabidopsis* OGDHC are viable (A. Nunes-Nesi, M. Lehmann and A. R. Fernie, unpublished work), and transgenic tomato plants inhibited in the expression of E1o subunit and exhibiting a reduction in OGDHC activity down to 35 % of wild-type levels have also been recovered (W. Araujo, A. Nunes-Nesi and A. R. Fernie, unpublished work). In both species the deficiency in expression and consequently in activity of OGDHC did not result in dramatic phenotypic changes. However, it is important to note that the level of analysis has only been cursory to date and is by no means satisfactory to exclude an important role of OGDHC in plant development and performance. It is interesting that the two genes for the E1o component, which have been recently revealed to code for the E1o isoforms in animals [145], are also found in *Arabidopsis* by Blast searches (AT5G65750 and AT3G55410). However, in view of the essential role of the well-known isoform of E1o in humans [10], this redundancy is unlikely to explain the modest effects of knocking down only one of these two genes for E1o in plants.

Despite the lack of direct genetic studies on OGDHC the wealth of data accumulating from proteomic, transcriptomic and metabolomic research affords us a range of interesting observations hinting towards an important role for the plant enzyme under a range of conditions [41,42,163–166]. Although very few studies have been focused towards modification of this enzyme under altered respiratory demand, plants exposed to various genetic or environmental perturbations have variously indicated important roles for OG and, by implication the OGDHC, in ammonium assimilation [23,163,164,166], gibberellin biosynthesis [167] and flavonoid and glucosinolate formation [168,169]. The metabolomic analysis following inhibition of plant OGDHC detailed in the section 'Involvement of plant OGDHC in nitrate assimilation' above is in close support of the studies for ammonium assimilation, but was not extensive enough to cover its proposed role in fueling and/or regulating biosynthesis of secondary metabolites.

Previous studies have additionally shown that the plant OGDHC is an early target of oxidative stress [162] and, in particular, by the cytotoxic lipid peroxidation product 4-hydroxynonen-2-al [170], and that the levels of OG rapidly increase on chemical inhibition of complex 1 [171]. Moreover, the incubation of leaf discs in dithiothreitol resulted in the depletion of the levels of organic acids in the upstream part of the tricarboxylic acid cycle from OGDHC and an accumulation of those downstream of the reaction catalysed by the complex [165]. The final study is in close accordance both with studies in mammalian and bacterial systems which revealed OGDHC to be regulated by cellular SH/-S-S and thioredoxin [35,39,40,172] and with the identification of the E2o subunit of the OGDHC in a proteomic survey that catalogues plant mitochondrial proteins capable of reacting with thioredoxins [41,42]. Detailed study of the recently isolated genotypes, displaying deficient expression of components of the OGDHC, will probably prove a highly effective means both to directly quantify importance of this enzyme within all of the processes defined above and even possibly to identify new functions of the complex in plants.

CONCLUSIONS

OGDHC has been observed to control respiration in several plant and animal systems, especially under conditions of increased energy demand. However, increased flux through OGDHC without appropriate elevation of its terminal substrate, NAD^+, may stimulate OGDHC-dependent ROS production from OG. This side reaction may signal a metabolic imbalance, further deteriorating homoeostasis when the condition persists. The states in which OGDHC-dependent ROS may contribute to cellular signalling and/or damage can be revealed by decreasing the ROS production using specific inhibitors of OGDHC such as the phosphonate analogues of OG *in vivo*. This approach has established the OGDHC contribution to the glutamate-induced ROS in neurons and to the wound-associated respiratory burst in plants. Another biologically relevant side reaction of OGDHC is the carboligase activity of its first thiamine-dependent component, which appears to be involved in glyoxylate scavenging.

Changes in cellular and tissue activity of OGDHC within a physiological range of conditions are met by complex metabolic

regulation of OGDHC by its multiple cofactors, substrates and products, as well as allosteric regulators, not involved in the reaction, such as ADP or AMP and Ca^{2+}. However, in the case of OGDHC impairments or upon metabolic switches (such as that to aerobic metabolism), biological systems elicit compensatory responses involving changes in expression of the OGDHC components. Expression of the first component, the thiamine-dependent OGDH, which limits the overall rate of OGDHC catalysis, is often increased under conditions causing OGDHC inactivation. Regulation of the expression of the terminal component, dihydrolipoyl dehydrogenase, may be an important mechanism by which to adjust the ROS-generating activity of OGDHC.

Upon the OGDHC block, the tricarboxylic acid cycle can be restored by a compensatory increased activity of the GABA shunt. However, such restoration causes significant shifts in cellular pools of amino acids. Moreover, the shunting cannot restore the calcium regulation of mitochondrial metabolism in animals, where OGDHC degrades the neurotransmitter precursor OG in a calcium-dependent manner. The OGDHC-dependent disturbances in calcium signalling and glutamate metabolism are obviously responsible for the defective development of the neural system and early death of the OGDHC mutants in animals, and for the association of the acquired OGDHC impairments with neurodegeneration. Plants do not possess the animal-like nervous system, nor the neurotransmission. The viability of the plant mutants of OGDHC therefore supports the idea that it is the essential connection between OGDHC, calcium and glutamate-dependent processes in the function and development of nervous tissue, which makes this complex indispensable for animal viability, but not for aerobic metabolism *per se*. Although these factors highlight the differences between the particular roles for the OGDHC reaction in species-specific networks, there are a great number of commonalities at the structural, enzymatic, regulatory and functional levels. The increasing abundance of mutants and the recent adoption of phosphonate analogues of OG will promote future development of the ideas outlined in the present review by a cross-kingdom comparison of the OGDHC networking through precise comparative experiments.

ACKNOWLEDGEMENTS

We gratefully acknowledge the help of Dr Dorel Guranda (Lomonosov Moscow State University, Moscow, Russia) and Mr Wagner Luis Araujos (Max-Planck-Institute for Plant Physiology, Potsdam–Golm, Federal Republic of Germany) in preparing Figures 2, 3 and 6. V.B. expresses her deep gratitude to the long-standing support of her co-operative work by the Alexander von Humboldt Foundation (Bonn, Germany)

FUNDING

Work on OGDHC in our laboratories was supported by the Deutsche Forshungsgemeinschaft [within the SFB (Sonderforschungsbereich) 428 programme]; and Russian Foundation of Basic Research [grant numbers 06-08-01441 and 09-04-90473).

REFERENCES

1 Kitano, H. (2002) Computational systems biology. Nature **420**, 206–210
2 Sweetlove, L. J., Last, R. L. and Fernie, A. R. (2003) Predictive metabolic engineering: a goal for systems biology. Plant Physiol. **132**, 420–425
3 Sweetlove, L. J., Fell, D. A. and Fernie, A. R. (2008) Getting to grips with the plant metabolic network. Biochem. J. **409**, 27–41
4 Geigenberger, P., Stitt, M. and Fernie, A. R. (2004) Metabolic control and regulation of the conversion of sucrose to starch in growing potato tubers. Plant Cell Environ. **27**, 655–673
5 Fell, D. A. (1997) Understanding the Control of Metabolism, Portland Press, London
6 Holms, H. (1996) Flux analysis and control of the central metabolic pathways in *Escherichia coli*. FEMS Microbiol. Rev. **19**, 85–116
7 Amsterdam, A., Nissen, R. M., Sun, Z., Swindell, E. C., Farrington, S. and Hopkins, N. (2004) Identification of 315 genes essential for early zebrafish development. Proc. Natl. Acad. Sci. U.S.A. **101**, 12792–12797
8 Almaas, E., Kovacs, B., Vicsek, T., Oltvai, Z. N. and Barabasi, A. L. (2004) Global organization of metabolic fluxes in the bacterium *Escherichia coli*. Nature **427**, 839–843
9 Johnson, M. T., Yang, H. S., Magnuson, T. and Patel, M. S. (1997) Targeted disruption of the murine dihydrolipoamide dehydrogenase gene (*Dld*) results in perigastrulation lethality. Proc. Natl. Acad. Sci. U.S.A. **94**, 14512–14517
10 Dunkelmann, R. J., Ebinger, F., Schulze, A., Wanders, R. J. A., Rating, D. and Mayatepek, E. (2000) 2-Ketoglutarate dehydrogenase deficiency with intermittent 2-ketoglutaric aciduria. Neuropediatrics **31**, 35–38
11 Guffon, N., Lopez-Mediavilla, C., Dumoulin, R., Mousson, B., Godinot, C., Carrier, H., Collombet, J. M., Divry, P., Mathieu, M. and Guibaud, P. (1993) 2-Ketoglutarate dehydrogenase deficiency, a rare cause of primary hyperlactataemia: report of a new case. Inherit. Metab. Dis. **16**, 821–830
12 Odièvre, M. H., Chretien, D., Munnich, A., Robinson, B. H., Dumoulin, R., Masmoudi, S., Kadhom, N., Rötig, A., Rustin, P. and Bonnefont, J. P. (2005) A novel mutation in the dihydrolipoamide dehydrogenase E3 subunit gene (*DLD*) resulting in an atypical form of α-ketoglutarate dehydrogenase deficiency. Hum. Mutat. **25**, 323–324
13 Bonnefont, J. P., Chretien, D., Rustin, P., Robinson, B., Vassault, A., Aupetit, J., Charpentier, C., Rabier, D., Saudubray, J. M. and Munnich, A. (1992) α-Ketoglutarate dehydrogenase deficiency presenting as congenital lactic acidosis. J. Pediatr. **121**, 255–258
14 Gibson, G. E., Blass, J. P., Beal, M. F. and Bunik, V. (2005) The α-ketoglutarate dehydrogenase complex: a mediator between mitochondria and oxidative stress in neurodegeneration. Mol. Neurobiol. **31**, 43–63
15 Bunik, V., Schloss, J. V., Pinto, J. T., Gibson, G. E. and Cooper, A. J. L. (2007) Enzyme-catalyzed side reactions with molecular oxygen may contribute to cell signalling and neurodegenerative diseases. Neurochem. Res. **32**, 871–891
16 Bunik, V. I. and Strumilo, S. (2009) Regulation of catalysis within cellular network: metabolic and signalling implications of the 2-oxoglutarate oxidative decarboxylation. Curr. Chem. Biol., in the press
17 He, W., Miao, F. J., Lin, D. C., Schwandner, R. T., Wang, Z., Gao, J., Chen, J. L., Tian, H. and Ling, L. (2004) Citric acid cycle intermediates as ligands for orphan G-protein-coupled receptors. Nature **429**, 188–193
18 McDonough, M. A., Li, V., Flashman, E., Chowdhury, R., Mohr, C., Liénard, B. M., Zondlo, J., Oldham, N. J., Clifton, I. J., Lewis, J. et al. (2006) Cellular oxygen sensing: crystal structure of hypoxia-inducible factor prolyl hydroxylase (PHD2). Proc. Natl. Acad. Sci. U.S.A. **103**, 9814–9819
19 Matsumoto, K., Imagawa, S., Obara, N., Suzuki, N., Takahashi, S., Nagasawa, T. and Yamamoto, M. (2006) 2-Oxoglutarate downregulates expression of vascular endothelial growth factor and erythropoietin through decreasing hypoxia-inducible factor-1α and inhibits angiogenesis. J. Cell Physiol. **209**, 333–340
20 Butow, R. A. and Avadhani, N. G. (2004) Mitochondrial signalling: the retrograde response. Mol. Cell **14**, 1–15
21 Laurent, S., Chen, H., Bedu, S., Ziarelli, F. and Zhang, C. C. (2005) Nonmetabololizable analogue of 2-oxoglutarate elicits heterocyst. Proc. Natl. Acad. Sci. U.S.A. **102**, 9907–9912
22 Chen, H., Laurent, S., Bédu, S., Ziarelli, F., Chen, H. L., Cheng, Y., Zhang, C. C. and Peng, L. (2006) Studying the signalling role of 2-oxoglutaric acid using analogues that mimic the ketone and ketal forms of 2-oxoglutaric acid. Chem. Biol. **13**, 849–856
23 Hodges, M. (2002) Enzyme redundancy and the importance of 2-oxoglutarate in plant ammonium assimilation. J. Exp. Bot. **53**, 905–916
24 Chen, Y. M., Ferrar, T. S., Lohmeier-Vogel, E. M., Morrice, N., Mizuno, Y., Berenger, B., Ng, K. K., Muench, D. G. and Moorhead, G. B. (2006) The PII signal transduction protein of *Arabidopsis thaliana* forms an arginine-regulated complex with plastid *N*-acetylglutamate kinase. J. Biol. Chem. **281**, 5726–5733
25 Daniel, J. and Danchin, A. (1986) 2-Ketoglutarate as a possible regulatory metabolite involved in cyclic AMP-dependent catabolite repression in *Escherichia coli* K12. Biochimie **68**, 303–310
26 Waskiewicz, D. E. and Hammes, G. G. (1980) Elementary steps in the reaction mechanism of the α-ketoglutarate dehydrogenase multienzyme complex from *Escherichia coli*: kinetics of succinylation and desuccinylation. Biochemistry **23**, 3136–3143
27 Graham, L. D., Packman, L. C. and Perham, R. N. (1989) Kinetics and specificity of reductive acylation of lipoyl domains from 2-oxo acid dehydrogenase multienzyme complexes. Biochemistry **28**, 1574–1581
28 Berg, A., Westphal, A. H., Bosma, H. J. and de Kok, A. (1998) Kinetics and specificity of reductive acylation of wild-type and mutated lipoyl domains of 2-oxo-acid dehydrogenase complexes from *Azotobacter vinelandii*. Eur. J. Biochem. **15**, 45–50

29 Bunik, V. and Sievers, C. (2002) Inactivation of the 2-oxo acid dehydrogenase complexes upon generation of intrinsic radical species. Eur. J. Biochem. **269**, 5004–5015
30 Schlossberg, M. A., Bloom, R. J., Richhert, D. A. and Westerfeld, W. W. (1970) Carboligase activity of α-ketoglutarate dehydrogenase. Biochemistry **59**, 1148–1153
31 O'Fallon, J. V. and Brosemer, R. W. (1977) Cellular localization of α-ketoglutarate:glyoxylate carboligase in rat tissues. Biochim. Biophys. Acta **499**, 321–328
32 Zündorf, G., Kahlert, S., Bunik, V. I. and Reiser, G. (2009) α-Ketoglutarate dehydrogenase contributes to production of reactive oxygen species in glutamate-stimulated hippocampal neurons *in situ*. Neuroscience **158**, 610–616
33 Sidhu, H., Gupta, R., Thind, S. K. and Nath, R. (1987) Oxalate metabolism in thiamine-deficient rats. Ann. Nutr. Metab. **31**, 354–361
34 Packer, L., Witt, E. H. and Tritschler, H. J. (1995) α-Lipoic acid as a biological antioxidant. Free Radical Biol. Med. **19**, 227–250
35 Bunik, V. (2003) 2-Oxo acid dehydrogenase complexes in redox regulation. Role of the lipoate residues and thioredoxin. Eur. J. Biochem. **270**, 1036–1042
36 Izard, T., Aevarsson, A., Allen, M. D., Westphal, A. H., Perham, R. N., de Kok, A. and Hol, W. G. (1999) Principles of quasi-equivalence and Euclidean geometry govern the assembly of cubic and dodecahedral cores of pyruvate dehydrogenase complexes. Proc. Natl. Acad. Sci. U.S.A. **96**, 1240–1245
37 Collins, J. H. and Reed, L. J. (1977) Acyl group and electron pair relay system – network of interacting lipoyl moieties in pyruvate and α-ketoglutarate dehydrogenase complexes from *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. **74**, 4223–4227
38 El Hanine Lmoumène, C., Conte, D., Jacquot, J.-P. and Houée-Levin, C. (2000) Redox properties of protein disulfide bond in oxidized thioredoxin and lysozyme: a pulse radiolysis study. Biochemistry **39**, 9295–9301
39 Bunik, V. (2000) Increased catalytic performance of the 2-oxoacid dehydrogenase complexes in the presence of thioredoxin, a thiol-disulfide oxidoreductase. J. Mol. Catal. B **8**, 165–174
40 Bunik, V., Raddatz, G., Lemaire, S., Meyer, Y., Jacquot, J.-P. and Bisswanger, H. (1999) Interaction of thioredoxins with target proteins: role of particular structural elements and electrostatic properties of thioredoxins in their interplay with 2-oxoacid dehydrogenase complexes. Protein Sci. **8**, 65–74
41 Balmer, Y., Vensel, W. H., Tanaka, C. K., Hurkman, W. J., Gelhaye, E., Rouhier, N., Jacquot, J. P., Manieri, W., Schuurmann, P., Droux, M. et al. (2004) Thioredoxin links redox to the regulation of fundamental processes of plant mitochondria. Proc. Natl. Acad. Sci. U.S.A. **101**, 2642–2647
42 Lemaire, S. D., Guillon, B., Le Marechal, P., Keryer, E., Miginiac-Maslow, M. and Decottignes, P. (2004) New thioredoxin targets in the unicellular photosynthetic eukaryote *Chlamydomonas reinhardtii*. Proc. Natl. Acad. Sci. U.S.A. **101**, 7475–7480
43 Zhou, J., Damdimopoulos, A. E., Spyrou, G. and Brüne, B. (2007) Thioredoxin 1 and thioredoxin 2 have opposed regulatory functions on hypoxia-inducible factor-1α. J. Biol. Chem. **282**, 7482–7490
44 Liang, C. C. (1962) Studies on experimental thiamine deficiency. 3. Glyoxylic acid, citric acid and tissue metabolism. Biochem. J. **85**, 38–44
45 Lawlis, V. B. and Roche, T. E. (1981) Regulation of bovine kidney α-ketoglutarate dehydrogenase complex by calcium ion and adenine nucleotides. Effect on $S_{0.5}$ for α-ketoglutarate. Biochemistry **20**, 2512–2518
46 Strumilo, S., Czygier, M., Kondracikowska, J., Dobrzyn, P. and Czerniecki, J. (2002) Kinetic and spectral investigation of allosteric interaction of coenzymes with 2-oxo acid dehydrogenase complexes. J. Mol. Struct. **614**, 221–226
47 Markiewicz, J. and Strumilo, S. A. (1995) Some regulatory properties of the 2-oxoglutarate dehydrogenase complex from European bison heart. Acta Biochim. Pol. **42**, 339–346
48 Bunik, V. I., Buneeva, O. A. and Gomazkova, V. S. (1990) Change in α-ketoglutarate dehydrogenase cooperative properties due to dihydrolipoate and NADH. FEBS Lett. **269**, 252–254
49 Gomazkova, V. S. and and Krasovskaya, O. E. (1979) Regulation of α-ketoglutarate dehydrogenase complex from pigeon breast muscle. Biokhimiya **44**, 1126–1136
50 Smith, C. M., Bryla, J. and Williamson, J. R. (1974) Regulation of mitochondrial ketoglutarate metabolism by product inhibition at α-ketoglutarate dehydrogenase. J. Biol. Chem. **249**, 1497–1505
51 Furuta, S., Shindo, Y. and Hashimoto, T. (1977) Purification and properties of pigeon breast muscle α-keto acid dehydrogenase complexes. J. Biochem. (Tokyo) **81**, 1839–1847
52 Lawlis, B. V. and Roche, T. E. (1981b) Inhibition of bovine kidney α-ketoglutarate dehydrogenase complex by reduced nicotinamide adenine dinucleotide in the presence or absence of calcium ion and effect of adenosine 5-diphosphate on reduced nicotinamide adenine dinucleotide inhibition. Biochemistry **20**, 2519–2524
53 Strumilo, S. A., Taranda, N. I. and Vinogradov, V. V. (1982) Peculiarities of the regulation of the adrenal oxoglutarate dehydrogenase complex by NADH and adenosine diphosphate. Biokhimiya **47**, 724–732
54 McCormack, J. G. and Denton, R. M. (1981) A comparative study of the regulation by Ca^{2+} of the activities of the 2-oxoglutarate dehydrogenase complex and NAD–isocitrate dehydrogenase from variety of sources. Biochem. J. **196**, 619–624
55 Wedding, R. T. and Black, M. K. (1971) Evidence for tighter binding of magnesium-thiamine pyrophosphate to α-ketoglutarate dehydrogenase when activated by adenosine monophosphate. J. Biol. Chem. **246**, 4097–4099
56 Craig, D. W. and Wedding, R. T. (1980) Regulation of the 2-oxoglutarate dehydrogenase–lipoate succinyltransferase complex from cauliflower by nucleotide: steady state kinetic studies. J. Biol. Chem. **255**, 5763–5768
57 Frank, R. A., Price, A. J., Northrop, F. D., Perham, R. N. and Luisi, B. F. (2007) Crystal structure of the E1 component of the *Escherichia coli* 2-oxoglutarate dehydrogenase multienzyme complex. J. Mol. Biol. **368**, 639–651
58 Kluger, R. and Pike, D. C. (1977) Active site generated analogues of reactive intermediates in enzymic reactions. Potent inhibition of pyruvate dehydrogenase by a phosphonate analogue of pyruvate. J. Am. Chem. Soc. **99**, 4504–4506
59 Kluger, R. and Pike, D. C. (1979) Chemical synthesis of a proposed enzyme-generated "reactive intermediate analogue" derived from thiamin diphosphate. Self-activation of pyruvate dehydrogenase by conversion of the analogue to its components. J. Am. Chem. Soc. **101**, 6425–6428
60 O'Brien, T. A., Kluger, R., Pike, D. C. and Gennis, R. B. (1980) Phosphonate analogues of pyruvate. Probes of substrate binding to pyruvate oxidase and other thiamin pyrophosphate-dependent decarboxylases. Biochim. Biophys. Acta **613**, 10–17
61 Arjunan, P., Sax, M., Brunskill, A., Chandrasekhar, K., Nemeria, N., Zhang, S., Jordan, F. and Furey, W. (2006) A thiamin-bound, pre-decarboxylation reaction intermediate analogue in the pyruvate dehydrogenase E1 subunit induces large scale disorder-to-order transformations in the enzyme and reveals novel structural features in the covalently bound adduct. J. Biol. Chem. **281**, 15296–15303
62 Kutter, S., Weiss, M. S., Wille, G., Golbik, R., Spinka, M. and König, S. (2009) Covalently bound substrate at the regulatory site of yeast pyruvate decarboxylases triggers allosteric enzyme activation. J. Biol. Chem. **284**, 12136–12144
63 Bunik, V. I., Biryukov, A. I. and Zhukov, Y. N. (1992) Inhibition of pigeon breast muscle α-ketoglutarate dehydrogenase by phosphonate analogueues of α-ketoglutarate. FEBS Lett. **303**, 197–201
64 Khomutov, R. M., Osipova, T. I. and Zhukov, Yu. N. (1977) Synthesis of *R*-ketophosphonic acids (translated from Russian) IzV. Akad. Nauk. SSSR Ser. Khim. **6**, 1391–1394
65 Biryukov, A. I., Bunik, V. I., Zhukov, Y. N., Khurs, E. N. and Khomutov, R. M. (1996) Succinyl phosphonate inhibits α-ketoglutarate oxidative decarboxylation, catalyzed by α-ketoglutarate dehydrogenase complexes from *E. coli* and pigeon breast muscle. FEBS Lett. **382**, 167–170
66 Bunik, V., Denton, T. T., Xu, H., Thompson, C. M., Cooper, A. J. L. and Gibson, G. E. (2005) Phosphonate analogues of α-ketoglutarate inhibit the activity of the α-ketoglutarate dehydrogenase complex isolated from brain and in cultured cells. Biochemistry **44**, 10552–10561
67 Araujo, W. L., Nunes-Nesi, A., Trenkamp, S., Bunik, V. I. and Fernie, A. R. (2008) Inhibition of 2-oxoglutarate dehydrogenase in potato tuber suggests the enzyme is limiting for respiration and confirms its importance in nitrogen assimilation. Plant Physiol. **148**, 1782–1796
68 Kabysheva, M. S., Storozhevykh, T. P., Pinelis, V. G. and Bunik, V. I. (2009) Synthetic regulators of the 2-oxoglutarate oxidative decarboxylation alleviate the glutamate excitotoxicity in cerebellar granule neurons. Biochem. Pharmacol. **77**, 1531–1540
69 Russel, III, R. R. and Taegtmeyer, H. (1992) Coenzyme A sequestration in rat hearts oxidising ketone bodies. J. Clin. Invest. **89**, 968–973
70 Rowan, A. N. and Newsholme, E. A. (1979) Changes in the contents of adenine nucleotides and intermediates of glycolysis and the citric acid cycle in flight muscle of the locust upon flight and their relationship to the control of the cycle. Biochem. J. **15**, 209–216
71 Bunik, V. I., Kabysheva, M. S., Klimuk, E. I., Storozhevykh, T. P. and Pinelis, V. G. (2009) Phosphono analogues of 2-oxoglutarate protect cerebellar granular neurons upon glutamate excitotoxicity. Ann. N.Y. Acad. Sci., in the press
72 Visser, W. F., van Roermund, C. W., Ijlst, L., Hellingwerf, K. J., Waterham, H. R. and Wanders, R. J. (2006) First identification of a 2-ketoglutarate/isocitrate transport system in mammalian peroxisomes and its characterization. Biochem. Biophys. Res. Commun. **348**, 1224–1231
73 Stipani, V., Cappello, A. R., Daddabbo, L., Natuzzi, D., Miniero, D. V., Stipani, I. and Palmieri, F. (2001) The mitochondrial oxoglutarate carrier: cysteine-scanning mutagenesis of transmembrane domain IV and sensitivity of Cys mutants to sulfhydryl reagents. Biochemistry **40**, 15805–15810
74 Butterworth, R. F. and Leong, D. K. (1996) Thiamine deficiency (Wernicke's) encephalopathy: pathophysiological mechanisms and development of positron emission tomography (PET) ligands. In Biochemistry and Physiology of Thiamin Diphosphate Enzymes (Bisswanger, H. and Schellenberger, A., eds), pp. 409–416, A.U.C. Intemann, Prien

75 Navarro, D., Zwingmann, C. and Butterworth, R. F. (2008) Region-selective alterations of glucose oxidation and amino acid synthesis in the thiamine-deficient rat brain: a re-evaluation using $^{1}H/^{13}C$ nuclear magnetic resonance spectroscopy. J. Neurochem. **106**, 603–612

76 Gibson, G., Nielsen, P., Mykytyn, V., Carlson, K. and Blass, J. (1989) Regionally selective alterations in enzymatic activities and metabolic fluxes during thiamin deficiency. Neurochem. Res. **14**, 17–24

77 Strumilo, S. A., Senkevich, S. B., Galitskii, E. A. and Vinogradov, V. V. (1983) Activity of thiamine pyrophosphate-dependent enzymes in the rats after oxythiamine administration. Biull. Eksp. Biol. Med. **96**, 42–44

78 Strumilo, S., Czygier, M. and Markiewicz, J. (1995) Different extent of inhibition of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase both containing endogenous thiamine pyrophosphate, by some anticoenzyme analogues. J. Enzyme Inhib. **10**, 65–72

79 Gomazkova, V. S., Stafeeva, O. A., Laufer, A. I. and and Severin, S. E. (1981) Investigation of the interaction of thiamine pyrophosphate with muscle α-ketoglutarate dehydrogenase. Dokl. Akad. Nauk. USSR **259**, 477–480

80 Bunik, V. and Pavlova, O. (1996) Interaction of 2-oxoglutarate dehydrogenase with 2-oxosubstrates and their structural analogues. In Biochemistry and Physiology of Thiamin Diphosphate Enzymes (Bisswanger, H. and Schellenberger, A., eds), pp. 367–373, A.U.C. Intemann, Prien

81 Liu, J. Y., Timm, D. E. and Hurley, T. D. (2006) Pyrithiamine as a substrate for thiamine pyrophosphokinase. J. Biol. Chem. **281**, 6601–6607

82 Parker, Jr, W. D., Haas, R., Stumpf, D. A., Parks, J., Eguren, L. A. and Jackson, C. (1984) Brain mitochondrial metabolism in experimental thiamine deficiency. Neurology **34**, 1477–1481

83 Cooney, G. J., Taegtmeyer, H. and Newsholme, E. A. (1981) Tricarboxylic acid cycle flux and enzyme activities in the isolated working rat heart. Biochem. J. **200**, 701–703

84 Kumar, M. J., Nicholls, D. G. and Andersen, J. K. (2003) Oxidative α-ketoglutarate dehydrogenase inhibition via subtle elevations in monoamine oxidase B levels results in loss of spare respiratory capacity: implications for Parkinson's disease. J. Biol. Chem. **278**, 46432–46439

85 Bunik, V. I. and Pavlova, O. G. (1997) Inactivation of α-ketoglutarate dehydrogenase during its enzymatic reaction. Biochemistry (Moscow) **62**, 973–982

86 Kahlert, S., Zündorf, G. and Reiser, G. (2005) Glutamate-mediated influx of extracellular Ca^{2+} is coupled with reactive oxygen species generation in cultured hippocampal neurons but not in astrocytes. Neurosci. Res. **79**, 262–271

87 Khodorov, B. I. (2004) Glutamate-induced deregulation of calcium homeostasis and mitochondrial dysfunction in mammalian central neurones. Prog. Biophys. Mol. Biol. **86**, 279–351

88 Hazell, A. S., Butterworth, R. F. and Hakim, A. M. (1993) Cerebral vulnerability is associated with selective increase in extracellular glutamate concentration in experimental thiamine deficiency. J. Neurochem. **61**, 1155–1158

89 Langlais, P. J. and Zhang, S. X. (1993) Extracellular glutamate is increased in thalamus during thiamine deficiency-induced lesions and is blocked by MK-801. J. Neurochem. **61**, 2175–2182

90 Santos, S., Gibson, G. G., Cooper, A. J. L., Denton, T. T., Thompson, C. M., Bunik, V. I., Alves, P. M. and Sonnewald, U. (2006) Inhibitors of α-ketoglutarate dehydrogenase complex alter [1-^{13}C]glucose and [U-^{13}C]glutamate metabolism in cerebellar granule neurons. J. Neurosci. Res. **83**, 450–458

91 Graf, A., Kabysheva M., Klimuk E., Trofimova, L., Dunaeva, T., Zundorf, G., Kahlert, S., Reiser, G., Storozhevykh, T., Pinelis, V., Sokolova, N. and Bunik, V. I. (2009) Role of 2-oxoglutarate dehydrogenase in brain pathologies involving glutamate neurotoxicity. J. Mol. Catal. B, doi:10.1016/j.molcatb.2009.02.016

92 Pardo, B., Contreras, L., Serrano, A., Ramos, M., Kobayashi, K., Lijima, M., Saheki, T. and Satrústegui, J. (2006) Essential role of Aralar in the transduction of small Ca^{2+} signals to neuronal mitochondria. Biol. Chem. **281**, 1039–1047

93 Ward, M. W., Huber, H. J., Weisova, P., Dussmann, H., Nicholls, D. G. and Prehn, J. H. (2007) Mitochondrial and plasma membrane potential of cultured cerebellar neurons during glutamate-induced necrosis, apoptosis and tolerance. Neuroscience **27**, 8238–8249

94 Veal, E. A., Day, A. M. and Morgan, B. A. (2007) Hydrogen peroxide sensing and signalling. Mol. Cell **26**, 1–13

95 Freeman, G. B., Nielsen, P. E. and Gibson, G. E. (1987) Effect of age on behavioral and enzymatic changes during thiamin deficiency. Neurobiol. Aging **8**, 429–434

96 Gibson, G. E., Perrino, P. and Dienel, G. (1984) Alterations of *in vivo* brain calcium homeostasis with aging. Age **7**, 148–149

97 Shi, Q., Karuppagounder, S. S., Xu, H., Pechman, D., Chen, H. and Gibson, G. E. (2007) Responses of the mitochondrial α-ketoglutarate dehydrogenase complex to thiamine deficiency may contribute to regional selective vulnerability. Neurochem. Int. **50**, 921–931

98 Adinolfi, A., Moratti, R., Olezza, S. and Ruffo, A. (1969) Control of the citric acid cycle by glyoxylate. The mechanism of inhibition of oxaloacetate dehydrogenase, isocitrate dehydrogenase and aconitate hydratase. Biochem. J. **114**, 513–518

99 Adinolfi, A., Olezza, S. and Ruffo, A. (1967) Inhibition of oxoglutarate dehydrogenase by glyoxylate and its condensation compounds. Biochem. J. **104**, 50P–51P

100 Ruffo, A., Testa, E., Pelizza, G. and Adinolfi, A. (1962) Control of citric acid cycle by glyoxylate 1. A new inhibitor of aconitase formed by condensation of glyoxylate with oxaloacetate. Biochem. J. **85**, 588–593

101 Jantzie, L. L., Cheung, P. Y., Obaid, L., Emara, M., Johnson, S. T., Bigam, D. L. and Todd, K. G. (2008) Persistant neurochemical changes in neonatal piglets after hypoxia–ischemia and resuscitation with 100 %, 21 % or 18 % oxygen. Resuscitation **77**, 111–120

102 Miyashita, K., Itoh, H. and Nakao, K. (2008) The neuroprotective and vasculo-neuro-regenerative roles of adrenomedullin in ischemic brain. Clin. Exp. Hypertens. **30**, 440–449

103 Hehre, D. A., Devia, C. J., Bancalari, E. and Suquihara, C. (2008) Brainstem amino acid neurotransmitters and ventilatory response to hypoxia in piglets. Pediatric Res. **63**, 46–50

104 Komuro, H. and Rakic, P. (1993) Modulation of neuronal migration by NMDA receptors. Science **260**, 95–97

105 Guerrini, L., Blasi, F. and Denisdonni, S. (1995) Synaptic activation of NF-κB by glutamate in cerebellar granule neurons *in vitro*. Proc. Natl. Acad. Sci. U.S.A. **92**, 9077–9081

106 Calingasan, N. Y., Ho, D. J., Wille, E. J., Campagna, M. V., Ruan, J., Dumont, M., Yang, L., Shi, Q., Gibson, G. E. and Beal, M. F. (2008) Influence of mitochondrial enzyme deficiency on adult neurogenesis in mouse models of neurodegenerative diseases. Neuroscience **153**, 986–996

107 Geigenberger, P., Fernie, A. R., Gibon, Y., Christ, M. and Stitt, M. (2000) Metabolic activity decreases as an adaptive response to low internal oxygen in growing potato tubers. Biol. Chem. **381**, 723–740

108 Kahl, G. (1974) Metabolism in plant storage tissue slices. Bot. Rev. **40**, 263–314

109 Roach, T., Ivanova, M., Beckett, R. P., Minibayeva, F. V., Green, I., Pritchard, H. W. and Kranner, I. (2008) An oxidative burst of superoxide in embryonic axes of recalcitrant sweet chestnut seeds as induced by excision and dessication. Physiol. Plant. **133**, 131–139

110 Minibayeva, F., Kolesnikov, O., Chasov, A., Beckett, R. P., Lüthje, S., Vylegzhanina, N., Buck, F. and Böttger, M. (2009) Wound-induced apoplastic peroxidase activities: their roles in the production and detoxification of reactive oxygen species. Plant Cell Environ. **32**, 497–508

111 Nunes-Nesi, A., Carrari, F., Lytovchenko, A., Smith, A. M. O., Ratcliffe, R. G., Sweetlove, L. J. and Fernie, A. R. (2005) Enhanced photosynthetic performance and growth as a consequence of decreasing mitochondrial malate dehydrogenase activity in transgenic tomato plants. Plant Physiol. **137**, 611–622

112 Nunes-Nesi, A., Carrari, F., Gibon, Y., Sulpice, R., Lytovchenko, A., Fisahn, J., Graham, J., Ratcliffe, R. G., Sweetlove, L. J. and Fernie, A. R. (2007) Deficiency of mitochondrial fumarase activity in tomato plants impairs photosynthesis via an effect on stomatal function. Plant J. **50**, 1093–1106

113 Sienkiewicz-Porzucek, A., Nunes-Nesi, A., Sulpice, R., Lisec, J., Centeno, D. C., Carillo, P., Leisse, A., Urbanczyk-Wochniak, E. and Fernie, A. R. (2008) Mild reductions in mitochondrial citrate synthase activity result in a compromised nitrate assimilation and reduced leaf pigmentation but have no effect on photosynthetic performance or growth. Plant Physiol. **147**, 115–27

114 Studart-Guimarães, C., Fait, A., Nunes-Nesi, A., Carrari, F., Usadel, B. and Fernie, A. R. (2007) Reduced expression of succinyl CoA ligase can be compensated for by an upregulation of the γ-amino-butyrate (GABA) shunt in illuminated tomato leaves. Plant Physiol. **145**, 626–639

115 Lemaitre, T., Urbanczyk-Wochniak, E., Flesch, V., Bismuth, E., Fernie, A. R. and Hodges, M. (2007) NAD-dependent isocitrate dehydrogenase mutants of *Arabidopsis* suggest the enzyme is not limiting for nitrogen assimilation. Plant Physiol. **144**, 1546–1558

116 Carrari, F., Nunes-Nesi, A., Gibon, Y., Lytovchenko, A., Loureiro, M. E. and Fernie, A. R. (2003) Reduced expression of aconitase results in an enhanced rate of photosynthesis and marked shifts in carbon partitioning in illuminated leaves of wild species tomato. Plant Physiol. **133**, 1322–1335

117 Canvin, D. T. and Beevers, H. (1961) Sucrose synthesis from acetate in the germinating castor bean: kinetics and pathway. J. Biol. Chem. **236**, 988–995

118 Hooks, M. A., Turner, J. E., Murphy, E. C., Johnston, K. A., Burr, S. and Jaroslawski, S. (2007) The *Arabidopsis* ALDP protein homologue COMATOSE is instrumental in peroxisomal acetate metabolism. Biochem. J. **406**, 399–406

119 Fernie, A. R., Krotzky, A., Trethewey, R. N. and Willmitzer, L. (2004) Metabolomics from diagnostics to systems biology. Nat. Rev. Mol. Cell. Biol. **9**, 673–679

120 Lisec, J., Schauer, N., Kopka, J., Willmitzer, L. and Fernie, A. R. (2006) Gas chromatorgraphy–mass spectrometry based metabolite profiling in plants. Nat. Protocols **1**, 387–396

121 Roessner, U., Luedemann, A., Brust, D., Fiehn, O., Linke, T., Willmitzer, L. and Fernie, A. R. (2001) Metabolite profiling allows comprehensive phenotyping of genetically or environmentally modified plant systems. Plant Cell **13**, 11–29

122 Roessner, U., Wochniak, E., Czechowski, T., Kolbe, A., Willmitzer, L. and Fernie, A. R. (2003) *De novo* amino acid biosynthesis in potato tubers is regulated by sucrose levels. Plant Physiol. **133**, 683–692

123 Dry, I. B. and Wiskich, J. T. (1985) Inhibition of 2-oxoglutarate oxidation in plant mitochondria by pyruvate. Biochem. Biophys. Res. Commun. **133**, 397–403

124 Cornu, S., Pireaux, J. C., Gerard, J. and Dizengremel, P. (1996) NAD(P)$^+$-dependent isocitrate dehydrogenases in mitochondria purified from *Picea abies* seedlings. Physiol. Plant. **96**, 312–318

125 Igamberdiev, A. U. and Gardeström, P. (2003) Regulation of NAD- and NADP-dependent isocitrate dehydrogenases by reduction levels of pyridine nucleotides in mitochondria and cytosol of pea leaves. Biochim. Biophys. Acta **1606**, 117–125

126 Picault, N., Palmieri, L., Pisano, I., Hodges, M. and Palmieri, F. (2002) Identification of a novel transporter for dicarboxylates and tricarboxylates in plant mitochondria: bacterial expression, reconstitution, functional characterization and tissue distribution. J. Biol. Chem. **277**, 24204–24211

127 Fait, A., Fromm, H., Walther, D., Galili, G. and Fernie, A. R. (2008) Highway or byway: the metabolic role of the GABA shunt in plants. Trends Plant Sci. **13**, 14–19

128 Hommes, N. G., Kurth, E. G., Sayavedra-Soto, L. A. and Arp, D. J. (2006) Disruption of *sucA*, which encodes the E1 subunit of α-ketoglutarate dehydrogenase, affects the survival of *Nitrosomonas europaea* in stationary phase. J. Bacteriol. **188**, 343–347

129 Taylor, I. J. and Anthony, C. (1976) A biochemical basis for obligate methylotrophy: properties of a mutant of *Pseudomonas* AM1 lacking 2-oxoglutarate dehydrogenase. J. Gen. Microbiol. **93**, 259–265

130 Repetto, B. and Tzagoloff, A. (1989) Structure and regulation of KGD1, the structural gene for yeast α-ketoglutarate dehydrogenase. Mol. Cell. Biol. **9**, 2695–2705

131 Repetto, B. and Tzagoloff, A. (1990) Structure and regulation of KGD2, the structural gene for yeat dihydrolipoyl transsuccinylase. Mol. Cell Biol. **10**, 4221–4232

132 Repetto, B. and Tzagoloff, A. (1991) *In vivo* assembly of yeast mitochondrial α-ketoglutarate dehydrogenase complex. Mol. Cell Biol. **11**, 3931–3939

133 Park, S. J., Chao, G. and Gunsalus, R. P. (1997) Aerobic regulation of the *sucABCD* genes of *Escherichia coli*, which encode α-ketoglutarate dehydrogenase and succinyl coenzyme A synthetase: roles of ArcA, Fnr and the upstream sdhCDAB promoter. J. Bacteriol. **179**, 4138–4142

134 Salmon, K. A., Hung, S. P., Steffen, N. R., Krupp, R., Baldi, P., Hatfield, G. W. and Gunsalus, R. P. (2005) Global gene expression profiling in *Escherichia coli* K12: effects of oxygen availability and ArcA. J. Biol. Chem. **280**, 15084–15096

135 Amarasin, C. R. and Davis, B. D. (1965) Regulation of α-ketoglutarate dehydrogenase formation in *Escherichia coli*. J. Biol. Chem. **240**, 3664–3669

136 Bunik, V., Westphal, A. H. and de Kok, A. (2000) Kinetic properties of the 2-oxoglutarate dehydrogenase complex from *Azotobacter vinelandii* evidence for the formation of a precatalytic complex with 2-oxoglutarate. Eur. J. Biochem. **267**, 3583–3591

137 Chistoserdova, L., Chen, S. W., Lapidus, A. and Lidstrom, M. E. (2003) Methylotrophy in *Methylobacterium extorquens* AM1 from a genomic point of view. J. Bacteriol. **185**, 2980–2987

138 Shiio, I. and Ujigawa-Takeda, K. (1980) Presence and regulation of α-ketoglutarate dehydrogenase complex in a glutamate-producing bacterium *Brevibacterium flavum*. Agric. Biol. Chem. **44**, 1897–1904

139 Schultz, C., Niebisch, A. and Gebel, L. (2007) Glutamate production by *Corynebacterium glutamicum*: dependence on the oxoglutarate dehydrogenase inhibitor protein OdhI and protein kinase PknG. Appl. Microbiol. Biotechnol. **76**, 691–700

140 Noda, Y., Berlett, B. S., Stadtman, E. R., Aponte, A., Morgan, M. and Shen, R. F. (2007) Identification of enzymes and regulatory properties in *Escherichia coli* that are oxidized under nitrogen, carbon or phosphate starvation. Proc. Natl. Acad. Sci. U.S.A. **104**, 18456–18460

141 Bettendorff, L., Wirtzfeld, B., Makarchikov, A. F., Mazzucchelli, G., Frederich, M., Gigliobianco, T., Gangolf, M., De Pauw, E., Argenot, L. and Wins, P. (2007) Discovery of a natural thiamine adenine nucleotide. Nat. Chem. Biol. **3**, 211–212

142 Lakaye, B., Wirtzfeld, B., Wins, P., Grisar, T. and Bettendorff, L. (2004) Thiamine triphosphate, a new signal required for optimal growth of *Escherichia coli* during amino acid starvation. J. Biol. Chem. **279**, 17142–17147

143 Meixner-Monori, B., Kubicek, C. P., Habison, A., Kubicek-Pranz, E. and Rohr, M. (1985) Presence and regulation of the α-ketoglutarate dehydrogenase multienzyme complex in the filamentous fungus *Aspergillus niger*. J. Bacteriol. **16**, 265–271

144 Koike, K. (1998) Cloning, structure, chromosomal localization and promoter analysis of human 2-oxoglutarate dehydrogenase gene. Biochim. Biophys. Acta **1385**, 373–384

145 Bunik, V. I., Kaehne, T., Degtyarev, D., Shcherbakova, T. and Reiser, G. (2008) Novel isoenzyme of 2-oxoglutarate dehydrogenease is identified in brain, but not in heart. FEBS J. **275**, 4990–5006

146 Nakano, K., Takase, C., Sakamoto, T., Nakagawa, S., Inazawa, J., Ohta, S. and Matuda, S. (1994) Isolation, characterization and structural organization of the gene and pseudogene for the dihydrolipoamide succinyltransferase component of the human 2-oxoglutarate dehydrogenase complex. Eur. J. Biochem. **224**, 179–189

147 Yang, H. S., Morris, J. I., Wang, Q., Korotchkina, L. G., Kwon, M. and Patel, M. S. (2001) Human dihydrolipoamide dehydrogenase gene transcription is mediated by cAMP-response element-like site and TACGAC direct repeat. Int. J. Biochem. Cell Biol. **33**, 902–913

148 McCartney, R. G., Rice, J. E., Sanderson, S., Bunik, V., Lindsay, H. and Lindsay, J. G. (1998) Subunit Interactions in the mammalian α-ketoglutarate dehydrogenase complex: Evidence for direct association of the α-ketoglutarate dehydrogenase (E1) and dihydrolipoamide dehydrogenase (E3) components. J. Biol. Chem. **273**, 24158–24164

149 Smolle, M., Prior, A. E., Brown, A. E., Cooper, A., Byron, O. and Lindsay, J. G. (2006) A new level of architectural complexity in the human pyruvate dehydrogenase complex. J. Biol. Chem. **281**, 19772–19780

150 Klivenyi, P., Starkov, A. A., Calingasan, N. Y., Gardian, G., Browne, S. E., Yang, L., Bubber, P., Gibson, G. E., Patel, M. S. and Beal, M. F. (2004) Mice deficient in dihydrolipoamide dehydrogenase show increased vulnerability to MPTP, malonate and 3-nitropropionic acid neurotoxicity. J. Neurochem. **88**, 1352–1360

151 Shi, Q., Chen, H. L., Xu, H. and Gibson, G. E. (2005) Reduction in the E2k subunit of the α-ketoglutarate dehydrogenase complex has effects independent of complex activity. J. Biol. Chem. **280**, 10888–10896

152 Habelhah, H., Laine, A., Erdjument-Bromage, H., Tempst, P., Gershwin, M. E., Bowtell, D. D. and Ronai, Z. (2004) Regulation of 2-oxoglutarate (α-ketoglutarate) dehydrogenase stability by the RING finger ubiquitin ligase Siah. J. Biol. Chem. **279**, 53782–53788

153 Kanamori, T., Nishimaki, K., Asoh, S., Ishibashi, Y., Takata, I., Kuwabara, T., Taira, K., Yamaguchi, H., Sugihara, S., Yamazaki, T. et al. (2003) Truncated product of the bifunctional *DLST* gene involved in biogenesis of the respiratory chain. EMBO J. **22**, 2913–2923

154 Matuda, S., Kodama, J., Goshi, N., Takase, C., Nakano, K., Nakagawa, S. and Ohta, S. (1997) A polypeptide derived from mitochondrial dihydrolipoamide succinyltransferase is located on the plasma membrane in skeletal muscle. Biochem. Biophys. Res. Commun. **241**, 151–156

155 Venkatraman, A., Landar, A., Davis, A. J., Chamlee, L., Sanderson, T., Kim, H., Page, G., Pompilius, M., Ballinger, S., Darley-Usmar, V. and Bailey, S. M. (2004) Modification of the mitochondrial proteome in response to the stress of ethanol-dependent hepatotoxicity. J. Biol. Chem. **279**, 22092–22101

156 Parkhomenko, Y. M., Donchenko, G. V., Pylypchuk, S. Y., Stepanenko, S. P., Chekhivska, L. I. and Klimenko, K. P. (2007) Characteristic metabolic disturbances in the rat tissues that were caused by long-term use of alcohol. Ukr. Biokhim. Zhurn. **79**, 61–69

157 Boluyt, M. O., Brevick, J. L., Rogers, D. S., Randall, M. J., Scalia, A. F. and Li, Z. B. (2006) Changes in the rat heart proteome induced by exercise training: increased abundance of heat shock protein hsp20. Proteomics **6**, 3154–3169

158 Thongboonkerd, V., Kanlaya, R., Sinchaikul, S., Parichatikanond, P., Chen, S. T. and Malasit, P. (2006) Proteomic identification of altered proteins in skeletal muscle during chronic potassium depletion: implications for hypokalemic myopathy. J. Proteome Res. **5**, 3326–3335

159 Ciszak, E. M., Korotchkina, L. G., Dominiak, P. M., Sidhu, S. and Patel, M. S. (2003) Structural basis for flip-flop action of thiamin pyrophosphate-dependent enzymes revealed by human pyruvate dehydrogenase. J. Biol. Chem. **278**, 21240–21246

160 Fernie, A. R., Carrari, F. and Sweetlove, L. J. (2004) Respiratory metabolism: glycolysis, the TCA cycle and mitochondrial electron transport, Curr. Opin. Plant Biol. **7**, 254–261

161 Sweetlove, L. J., Fait, A., Nunes-Nesi, A, Williams, T. and Fernie, A. R. (2007) The mitochondria. An integration point of cellular metabolism and signalling. Crit. Rev. Plant Sci. **26**, 17–43

162 Sweetlove, L. J., Heazelwood, J. L., Herald, V., Holtzapffel, R., Day, D. A., Leaver, C. J. and Millar, A. H. (2002) The impact of oxidative stress on *Arabidopsis* mitochondria. Plant J. **32**, 891–904

163 Dutilleul, C., Lelarge, C., Prioul, J. L., De Paepe, R., Foyer, C. H. and Noctor, G. (2005) Mitochondria-driven changes in leaf NAD status exert a crucial influence on the control of nitrate assimilation and the integration of carbon and nitrogen metabolism. Plant Physiol. **139**, 64–78

164 Fritz, C., Mueler, C., Matt, P., Feil, R. and Stitt, M. (2006) Impact of the C–N status on the amino acid profile in tobacco source leaves. Plant Cell Environ. **29**, 2055–2076

165 Kolbe, A., Oliver, S. N., Fernie, A. R., Stitt, M., van Dongen, J. T. and Geigenberger, P. (2006) Combined transcript and metabolite profiling of *Arabidopsis* leaves reveals fundamental affects of the thiol-dislphide status on plant metabolism. Plant Physiol. **141**, 412–422

166 Schneidereit, J., Häusler, R. E., Fiene, G., Kaiser, W. M. and Weber, A. P. M. (2006) Antisense repression reveals a crucial role of the plastidic 2-oxoglutarate/malate translocator DiT1 at the interface between carbon and nitrogen metabolism. Plant J. **45**, 206–224

167 Hedden, P. and Kamiya, Y. (1997) Gibberellin biosynthesis: enzymes, genes and their regulation. Annu. Rev. Plant Physiol. Plant Mol. Biol. **48**, 431–460
168 Saito, K., Kobayashi, M., Tanaka, Y., Gong, Z. and Yamazaki, M. (1999) Direct evidence for anthocyanidin synthase as a 2-oxoglutarate dependent dioxygenase: molecular cloning and functional expression of a red form of *Perilla frutescens*. Plant J. **17**, 181–189
169 Kliebenstein, D. J., Lambrix, V. M., Reichelt, M., Gershezon, J. and Mitchell-Olds, T (2001) Gene-duplication in the diversification of secondary metabolism: tandem 2-oxoglutarate-dependent dioxygenases control glucosinolate biosynthesis in *Arabidopsis*. Plant Cell **13**, 681–693

Received 12 May 2009/10 July 2009; accepted 10 July 2009
Published on the Internet 27 August 2009, doi:10.1042/BJ20090722

170 Millar, A. H. and Leaver, C. J. (2000) The cytotoxic lipid peroxidation product, 4-hydroxy-2-nonenol, specifically inhibits decarboxylating dehydrogenases in the matrix of plant mitochondria. FEBS Lett. **481**, 117–121
171 Garmier, M., Carroll, A. J., Delannoy, E., Vallet, C., Day, D. A., Small, I. D. and Millar, A. H. (2008) Complex I dysfunction redirects cellular and mitochondrial metabolism in *Arabidopsis*. Plant Physiol. **148**, 1324–1341
172 Raddatz, G., Kruft, V. and Bunik, V. (2000) Structural determinants for the efficient and specific interaction of thioredoxin with 2-oxoacid dehydrogenase complexes. Appl. Biochem. Biotechnol. **88**, 77–96