International Journal of Biochemistry and Cell Biology 115 (2019) 105580

Contents lists available at ScienceDirect

International Journal of Biochemistry and Cell Biology

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

Organelles in focus

Succinate in ischemia: Where does it come from?

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ARTICLE INFO

Keywords:
Fumarate
Hypoxia
Anoxia
Substrate-level phosphorylation
TCA cycle
Succinate dehydrogenase

ABSTRACT

During tissue ischemia succinate accumulates. Herein, literature spanning the past nine decades is reviewed leaning towards the far greater role of Krebs cycle's canonical activity yielding succinate through α-ketoglutarate - > succinyl-CoA - > succinate even in hypoxia, as opposed to reversal of succinate dehydrogenase. Furthermore, the concepts of i) a diode-like property of succinate dehydrogenase rendering it difficult to reverse, and ii) the absence of mammalian mitochondrial quinones exhibiting redox potentials in the [-60, -80] mV range needed for fumarate reduction, are discussed. Finally, it is emphasized that a "fumarate reductase" enzyme entity reducing fumarate to succinate found in some bacteria and lower eukaryotes remains to be discovered in mammalian mitochondria.

1. Introduction

That muscle contains succinate as an endogenous metabolite was first reported by Einbeck in 1914 (Einbeck, 1914), disproving views suggested by Wolff in 1904 (referenced in Moyle, 1924) that it is always a product of putrefaction. The discovery that muscle converts glutamate and aspartate to succinate, fumarate and malate during anaerobiosis is credited to Dorothy Mary Moyle Moyle, 1924; Needham (1930) in the 1920s (Needham DM and Moyle DM is the same person: she assumed the family name of her spouse, Joseph Needham). She further postulated that during anaerobic conditions, succinate is not formed reversibly from fumarate nor malate (Needham, 1927). Observations on the same line of research, *i.e.,* succinate is formed from pyruvate and/or α-ketoglutarate during anaerobiosis have been reported by Toenniessen and Brinkmann in 1930 (Toenniessen, 1930) and six years later by Weil-Malherbe (1936) and even Krebs (1936)!

The present mini-review focuses on i) literature appearing within the past 95 years regarding the origin of succinate in tissue ischemia/hypoxia/anoxia, ii) the difficulty of succinate dehydrogenase (SDH) operating in reverse due to an inherent diode-like property and the lack of suitable quinones in mammalian mitochondria for reduction of fumarate, iii) the pitfalls regarding data interpretation implicating SDH directionality and especially when using malonate in whole tissues or intact cells and iv) exerting caution on the concept of a mammalian mitochondrial "fumarate reductase" enzyme entity, known to be expressed only in some bacteria and lower eukaryotes. The roles of succinate as a signal and downstream pathological implications stemming from its accumulation are reviewed elsewhere (Tretter et al., 2016; Mills and O'Neill, 2014; Murphy and O'Neill, 2018; Grimolizzi and Arranz, 2018; Benit et al., 2014; Andrienko et al., 2017; Pell et al., 2016; Mithieux, 2018).

2. Succinate as a substrate or product: biochemical pathways

In humans, succinate can be substrate or product in 31 reactions (for comparison: 51 in mammals), summarized in Table 1 (assembled from BRENDA database, www.brenda-enzymes.org, (Jeske et al., 2019)). These reactions take place within different subcellular compartments, each contributing in altering tissue succinate concentration to a variable extent; thus, they all need to be taken into consideration if succinate is quantified by metabolomic analysis from whole tissue extracts or cells. However, the reactions anticipated to play a significant role in altering tissue succinate concentration within minutes of ischemia/hypoxia are depicted in Fig. 1, all taking place within mitochondria. The thickness and size of the arrows imply flux amplitude based on literature reviewed in sections 3, 3.1, 5 and 5.1. Succinate formed through ketone body metabolism or the GABA shunt is not expected to play a significant role (omitted for clarity), see also (Tretter et al., 2016). The rapid loss of accumulated succinate upon restoration of blood flow is better explained by its efflux through the monocarboxylate transporter (MCT1) rather than its catabolism (Andrienko et al., 2017).

3. In ischemia, succinate originates mostly from canonical TCA activity

Following the reports by Moyle-Needham in the 20 s (Moyle, 1924; Needham, 1927, 1930), Toenniessen (1930), Weil-Malherbe (1936),

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https://doi.org/10.1016/j.biocel.2019.105580
Received 1 May 2019; Received in revised form 30 July 2019; Accepted 2 August 2019
Available online 05 August 2019
1357-2725/

Table 1
Reactions in which succinate is substrate or product, in humans.

Enzyme	E.C. number	Reaction catalyzed
succinate dehydrogenase	EC 1.3.5.1	succinate + ubiquinone = fumarate + ubiquinol succinate + FAD = fumarate + $FADH_2$
succinate-hydroxymethylglutarate CoA-transferase	EC 2.8.3.13	3-hydroxy-3-methylglutaryl-CoA + succinate = 3-hydroxy-3-methylglutarate + succinyl-CoA
succinate-CoA ligase (GDP-forming)	EC 6.2.1.4	GTP + succinate + CoA = GDP + phosphate + succinyl-CoA
succinate-CoA ligase (ADP-forming)	EC 6.2.1.5	ATP + succinate + CoA = ADP + phosphate + succinyl-CoA
gamma-butyrobetaine dioxygenase	EC 1.14.11.1	gamma-butyrobetaine + α-ketoglutarate + O_2 = carnitine + succinate + CO_2 (the enzyme also catalyzes other reactions involving succinate)
peptide-aspartate beta-dioxygenase	EC 1.14.11.16	peptide L-aspartate + α-ketoglutarate + O_2 = peptide 3-hydroxy-L-aspartate + succinate + CO_2 (the enzyme also catalyzes other reactions involving succinate)
taurine dioxygenase	EC 1.14.11.17	taurine + α-ketoglutarate + O_2 = succinate + CO_2 + aminoethanol + sulfite
phytanoyl-CoA dioxygenase	EC 1.14.11.18	phytanoyl-CoA + α-ketoglutarate + O_2 = alpha-hydroxyphytanoyl-CoA + succinate + CO_2 (the enzyme also catalyzes other reactions involving succinate)
procollagen-proline 4-dioxygenase	EC 1.14.11.2	proline containing peptide + α-ketoglutarate + O_2 = 4-hydroxyproline containing peptide + succinate + CO_2 (the enzyme also catalyzes other reactions involving succinate)
[histone-H3]-lysine-36 demethylase	EC 1.14.11.27	protein N6,N6-dimethyl-L-lysine + α-ketoglutarate + O_2 = protein N6-methyl-L-lysine + succinate + formaldehyde + CO_2 (the enzyme also catalyzes other reactions involving succinate)
hypoxia-inducible factor-proline dioxygenase	EC 1.14.11.29	hypoxia-inducible factor-L-proline + α-ketoglutarate + O_2 = hypoxia-inducible factor-(4R)-4-hydroxy-L-proline + succinate + CO_2 (the enzyme also catalyzes other reactions involving succinate)
hypoxia-inducible factor-asparagine dioxygenase	EC 1.14.11.30	hypoxia-inducible factor-L-asparagine + α-ketoglutarate + O_2 = hypoxia-inducible factor-(3S)-3-hydroxy-L-asparagine + succinate + CO_2 (the enzyme also catalyzes other reactions involving succinate)
DNA oxidative demethylase	EC 1.14.11.33	DNA-1-methylguanine + α-ketoglutarate + O_2 = DNA-guanine + formaldehyde + succinate + CO_2 (the enzyme also catalyzes other reactions involving succinate)
procollagen-lysine 5-dioxygenase	EC 1.14.11.4	peptidyl-L-lysine + α-ketoglutarate + O_2 = peptidyl-5-hydroxy-L-lysine + succinate + CO_2 (the enzyme also catalyzes other reactions involving succinate)
tRNAPhe (7-(3-amino-3-carboxypropyl)wyosine37-C2)-hydroxylase	EC 1.14.11.42	7-(3-amino-3-carboxypropyl)wyosine37 in tRNAPhe + α-ketoglutarate + O_2 = 7-(2-hydroxy-3-amino-3-carboxypropyl)wyosine37 in tRNAPhe + succinate + CO_2
DNA N6-methyladenine demethylase	EC 1.14.11.51	N6-methyladenine in DNA + α-ketoglutarate + O_2 = adenine in DNA + formaldehyde + succinate + CO_2
mRNA N6-methyladenine demethylase	EC 1.14.11.53	N6-methyladenine in mRNA + α-ketoglutarate + O_2 = adenine in mRNA + formaldehyde + succinate + CO_2 (the enzyme also catalyzes other reactions involving succinate)
mRNA N1-methyladenine demethylase	EC 1.14.11.54	N1-methyladenine in mRNA + α-ketoglutarate + O_2 = adenine in mRNA + formaldehyde + succinate + CO_2 (the enzyme also catalyzes other reactions involving succinate)
procollagen-proline 3-dioxygenase	EC 1.14.11.7	(Gly-L-Pro-L-4-hydroxyproline)5 + α-ketoglutarate + O_2 = (Gly-trans-3-hydroxy-L-Pro-trans-4-hydrox-L-Pro)5 + succinate + CO_2 (the enzyme also catalyzes other reactions involving succinate)
trimethyllysine dioxygenase	EC 1.14.11.8	N6,N6,N6-trimethyl-L-lysine + α-ketoglutarate + O_2 = 3-hydroxy-N6,N6,N6-trimethyl-L-lysine + succinate + CO_2 (the enzyme also catalyzes other reactions involving succinate)
[histone-H3]-lysine-9-demethylase	EC 1.14.11.B1	[histone H3]-N6,N6,N6-trimethyl-L-lysine4 + α-ketoglutarate + O_2= [histone H3]-L-lysine4 + succinate + formaldehyde + CO_2 (the enzyme also catalyzes other reactions involving succinate)
[histone-H3]-lysine-4-demethylase	EC 1.14.11.B2	protein 6-N,6-N-dimethyl-L-lysine + α-ketoglutarate + O_2 = protein 6-N-methyl-L-lysine + succinate + formaldehyde + CO_2 (the enzyme also catalyzes other reactions involving succinate)
5,6-dihydroxyindole-2-carboxylic acid oxidase	EC 1.14.18.B1	histone H3 N6,N6,N6-trimethyl-L-lysine27 + α-ketoglutarate + O_2 = histone H3 N6,N6-dimethyl-L-lysine27 + succinate + formaldehyde + CO_2 (the enzyme also catalyzes other reactions involving succinate)
succinate-semialdehyde dehydrogenase	EC 1.2.1.24	succinate semialdehyde + $NAD(P)^+$ + H_2O = succinate + NAD(P)H
dihydroorotate dehydrogenase[a]	EC 1.3.98.1	L-dihydroorotate + fumarate = orotate + succinate
3-oxoacid CoA-transferase	EC 2.8.3.5	succinyl-CoA + a 3-oxo acid = succinate + a 3-oxoacyl-CoA (3-oxo acid can be acetoacetate or 3-hydroxybutyrate)
cholinesterase	EC 3.1.1.8	succinyl(thio)choline + H_2O = succinate + (thio)choline
S-succinylglutathione hydrolase	EC 3.1.2.13	S-succinylglutathione + H_2O = glutathione + succinate
acyl-CoA hydrolase	EC 3.1.2.20	succinyl-CoA + H_2O = CoA + succinate
hydroxyacylglutathione hydrolase	EC 3.1.2.6	S-succinylglutathione + H_2O = glutathione + succinate (same as EC 3.1.2.13)
acylphosphatase	EC 3.6.1.7	succinoyl phosphate + H_2O = succinate + phosphate

[a] This enzyme may not perform this reaction in human mitochondria because mammalian dihydroorotate dehydrogenase is a "Type 2″ enzyme, thus, its reaction mechanism is different than the "Type 1″ found in BRENDA database.

and Krebs (1936) in the 30 s -all showing that succinate accumulates in anoxic tissues originating from canonical TCA cycle activity- and the hiatus in research due to the second World War, the topic was re-addressed by Hunter in 1949 (Hunter, 1949). He showed that in anoxic rat kidney and liver, α-ketoglutarate is oxidized to succinate and CO_2, while oxaloacetate is reduced to malate; furthermore, α-ketoglutarate oxidation and creation of high energy phosphate bonds were equimolarly coupled. Essentially, he was describing that α-ketoglutarate was being transformed to succinyl-CoA using NAD^+ coming from mitochondrial malate dehydrogenase (MDH2) reversal, and that succinyl-CoA was becoming succinate forming high-energy phosphates through mitochondrial substrate-level phosphorylation (mSLP) (Chinopoulos et al., 2010), see Fig. 1.

A flurry of similar findings using different substrate combinations, methods of ischemia/hypoxia/anoxia and multitude of tissues and preparations were reported every few years or so to date, reaching the same conclusion: in ischemia/hypoxia/anoxia, α-ketoglutarate and upstream metabolites oxidize to succinate and this is coupled to equimolar production of high energy phosphate bonds, reflecting mSLP: (Chinopoulos et al., 2010; Penney and Cascarano, 1970; Gailis and Benmouyal, 1973; Hochachka et al., 1975; Taegtmeyer et al., 1977; Taegtmeyer, 1978; Sanborn et al., 1979; Freminet et al., 1980; Pisarenko et al., 1983; Peuhkurinen et al., 1983; Pisarenko et al., 1985; Bittl and Shine, 1983; Gronow and Cohen, 1984; Matsuoka et al., 1986; Pisarenko et al., 1986; Weinberg et al., 2000; Kiss et al., 2013, 2014; Nemeth et al., 2016; Kacso et al., 2016; Chinopoulos and Seyfried, 2018; Ravasz et al., 2018; Kohlhauer et al., 2018; Bui et al., 2019). Recently, proof that succinate accumulates in cardiac ischemia *via* α-

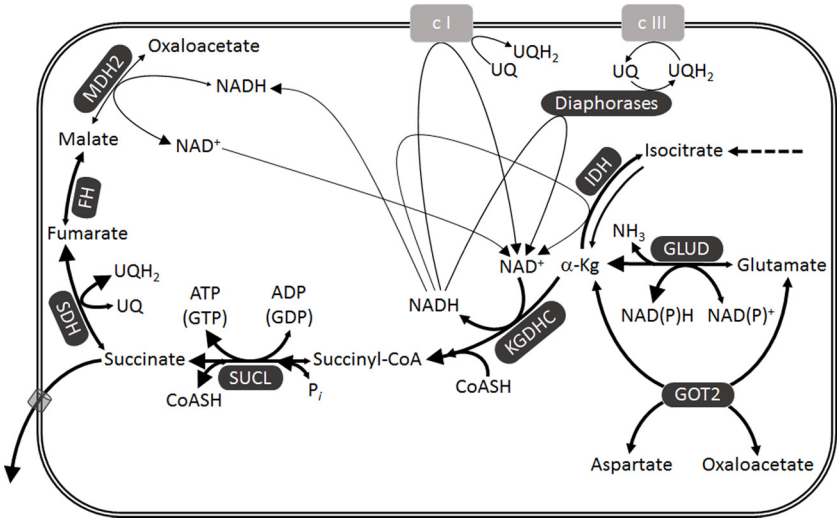

Fig. 1. Reactions altering succinate concentration in the mitochondrial matrix. α-Kg: α-ketoglutarate; c I: complex I of the respiratory chain; c III: complex III of the respiratory chain; FH: fumarate hydratase; GLUD: glutamate dehydrogenase; GOT2: aspartate aminotransferase isoform 2 (mitochondrial); KGDHC: α-ketoglutarate dehydrogenase complex; MDH2: malate dehydrogenase isoform 2 (mitochondrial); SDH: succinate dehydrogenase; SUCL: succinate-CoA ligase; UQ: Ubiquinone; UQH_2: Ubiquinol. Succinate exits mitochondria probably through both MCT1 and the dicarboxylate transporter.

ketoglutarate - > succinyl-CoA - > succinate came through metabolomic analysis using ^{13}C-labeling, performed by the Brookes lab (Zhang et al., 2018).

3.1. *If succinate originates from canonical TCA activity during ischemia, where does NAD$^+$ (needed for the α-ketoglutarate dehydrogenase complex) come from?*

As mentioned above, Hunter reported that in anoxic rat kidney and liver, α-ketoglutarate is oxidized quantitatively to succinate and CO_2 while oxaloacetate is reduced to malate, implying NAD$^+$ recycling through MDH2 (operating in reverse) and α-ketoglutarate dehydrogenase complex (KGDHC) (Hunter, 1949), see Fig. 1. This is not far-fetched, mindful that MDH2 is strongly favored towards reduction of oxaloacetate due to a large positive change of free energy (ΔG) of the reaction (+28.04 kJ/mol, Chinopoulos, 2013); *in organello*, it proceeds towards oxaloacetate because the larger ΔG in the negative range (-36.6 kj/mol Chinopoulos, 2013) of citrate synthase pulls the reaction along and keeps oxaloacetate concentration at a very low level. Other sources of NAD$^+$ have been proposed: the malate/aspartate shuttle operating in reverse -a possibility entertained in (Chouchani et al., 2014)- and/or matrix diaphorases: at least one of them is NQO1 which is known to provide NAD$^+$ to KGDHC during mitochondrial respiratory arrest, (Kiss et al., 2014; Ravasz et al., 2018). Another possibility is residual activity of complex I; although this has not been specifically addressed, data from (Zhang et al., 2018) support this, because rotenone, a specific inhibitor of complex I yielded *less* succinate during anoxia. Interestingly, in the 1967 report by Hoberman and Prosky that favor succinate formation by reduction of fumarate through mostly SDH reversal, inclusion of rotenone in the Ringer's liver perfusate resulting in an incomplete anaerobiosis also yielded *less* succinate, than in the absence of rotenone (Hoberman and Prosky, 1967). This could mean that complex I was still performing NADH oxidation –albeit small- but to an extent sufficient for KGDHC to support mSLP. Finally, NAD$^+$-dependent isocitrate dehydrogenase was also shown to operate in reverse towards NAD$^+$ formation, a process driven by acidic pH (Nadtochiy et al., 2016); relevant to this, it is important to emphasize that acidic pH is a hallmark of ischemia/hypoxia (Rouslin and Broge, 1989; Katsura et al., 1991).

4. Limitations in reverse operation of SDH due to a diode-like property *and* absence of suitable quinones in mammalian mitochondria

The reaction catalyzed by SDH exhibits a ΔG of -0.59 kj/mol (Chinopoulos, 2013), thus, from the thermodynamic point of view it is reversible. Indeed, SDH reversibility was shown by Thunberg in 1925 (Thunberg, 1925); this was re-addressed by Massey and Singer in 1957 (Massey and Singer, 1957a). More specifically, they used specific electron carriers in order to demonstrate reversibility of isolated SDH (Massey and Singer, 1957b). Likewise, by using such carriers, the group of Cecchini showed that the reaction catalyzed by mammalian SDH in reverse is ˜40 times slower than that in forward mode (Maklashina et al., 2018). There are two factors contributing to the difficulty of SDH in reducing fumarate: firstly, SDH behaves like a diode (Sucheta et al., 1992), *i.e.*, reduction of fumarate abruptly slows to a diminished catalytic rate below a redox potential of ˜ -60 −80 mV, despite an increase in the driving force. The diode-like property (also known as *tunnel diode effect* due to its similarity to an electronic device exhibiting the property of negative resistance) enforcing kinetics over thermodynamics has been thoroughly examined by Fraser Armstrong, Brian Ackrell, Judy Hirst and colleagues (Pershad et al., 1999; Hirst et al., 1997, 1996) using cyclic voltammetry. By adsorbing purified SDH A/B subunits on a graphite electrode immersed in an equimolar mixture of succinate and fumarate and changing the electrode potential that mimicked the redox potential of the quinone pool, in a cyclic manner (pH 7, 38 °C), they obtained voltammograms as depicted in Fig. 2. When the applied potential was in the positive range, succinate oxidation was observed. When the potential was in the negative range, fumarate reduction commenced at an appreciable rate only in the range of -60 −80 mV. At potentials more negative than −80 mV fumarate reduction was severely depressed. This suggests that SDH can only reduce fumarate if the redox potential of the quinone pool falls within a narrow range [-60, -80] mV, an observation evoking the second argument regarding the difficulty of SDH in reducing fumarate in mammalian mitochondria: ubiquinones found in mammalian mitochondria exhibit redox potentials of > +45 mV (Urban and Klingenberg, 1969). Thus, unless quinones exhibiting redox potentials in the [-60, -80] mV range exist –and yet to be discovered- the severely diminished capacity of SDH reducing fumarate in mammalian mitochondria can be partially explained by this fact alone. The explanation behind the diode-like property of SDH has been attributed to reduction of FAD and that a conformational change may occur upon formation of $FADH_2$, (Hirst et al., 1996); this latter mechanism has been proposed to represent an evolutionary adaptation to aerobic metabolism (Ackrell et al., 1993).

5. Succinate originating from SDH reversal: reports and pitfalls in data interpretation

Just as succinate has been widely reported to originate from

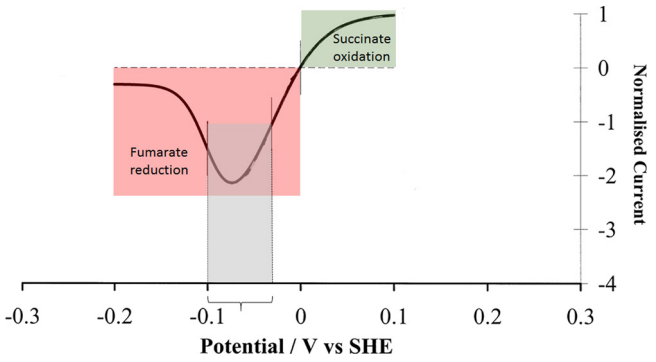

Fig. 2. Catalytic profile for SDH (adsorbed on a graphite electrode) acting on a 1:1 succinate/fumarate mixture (pH 7.0, 38°C) as a function of applied potential (potential in volts *vs* that of Standard Hydrogen Electrode [SHE]), mimicking the redox potential of the quinone pool. Modified from (Pershad et al., 1999), distributed under a Creative Commons license. Green rectangle represents quinone redox potential values that allow succinate oxidation. Red rectangle represents quinone redox potential values that allow fumarate reduction. Grey area represents the range of quinone redox potential values in which SDH performs reduction of fumarate at an appreciable rate. Note that mammalian mitochondria harbor ubiquinone that exhibits a redox potential of > +45 mV, (Urban and Klingenberg, 1969). Thus, from the kinetic point of view, reduction of fumarate to succinate by mammalian SDH *in organello*, is severely restricted. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

canonical TCA cycle activity, contribution from SDH reversal has also been methodically scrutinized. During ischemia, succinate was unequivocally shown to originate not only through α-ketoglutarate, but also from fumarate or upstream metabolites (see Fig. 1); however, all studies agreed that the extent of this ranges from 2% to 15%: (Hoberman and Prosky, 1967; Cascarano et al., 1968; Taegtmeyer et al., 1977; Taegtmeyer, 1978; ; Hohl et al., 1987; Wiesner et al., 1988; Kita et al., 1988; Laplante et al., 1997; Zhang et al., 2018). Some of these reports evoked the argument of a fumarate reductase system, in which fumarate is reduced to succinate by SDH reversal and this is coupled to NADH oxidation and ATP production also involving complex I and cytochrome *b*. This fumarate reductase *system* (not to be confused with the fumarate reductase *enzyme* found in some bacteria and lower eukaryotes addressed in section 5.1) has been originally proposed by Slater in 1950 (Slater, 1950), later refined in the 60′s and 70′s by Sanadi and Fluharty (1963); Haas (1964) and Wilson and Cascarano (1970). The contribution of cytochrome *b*has been subsequently refuted by the group of Vinogradov (Grivennikova et al., 1993). The concept of a fumarate reductase system in mammalian mitochondria –mostly in submitochondrial particles- gained momentum because of the near simultaneous discovery of the genuine fumarate reductase enzyme in helminths in 1969 (De Zoeten and Tipker, 1969), fueling the erroneous idea that the enzyme exists in all types of mitochondria. Furthermore, the possibility of ATP production from mSLP was not known at that time, as it was believed that succinate-CoA ligase could generate only guanine nucleotides, now known to be incorrect (Johnson et al., 1998; Lambeth et al., 2004); the discovery of mitochondrial nucleotide diphosphate kinases transferring a high-energy phosphate bond from one type of nucleotide to another was reported in 1962 (Chiga and Plaut, 1962) and re-addressed in 1967 (Goffeau et al., 1967) and 1969 (Colomb et al., 1969), thus, it is not known if this information was brought to these authors' attention as there is no cross-reference. Finally, the reduction of fumarate through SDH reversal -whether in isolation or in submitochondrial particles- could only be shown by using artificial electron carriers, discussed in (Grivennikova et al., 1993). Mindful that the only reaction in the matrix capable of generating ATP during respiratory arrest is that catalyzed by succinate-CoA ligase (Chinopoulos, 2011a; Chinopoulos et al., 2010), this fumarate reductase system could only be a combination of i) minor SDH reversal, ii) residual complex I activity oxidizing NADH or some other reaction (see section 3.1) and iii) mSLP yielding ATP. The lack of a genuine fumarate reductase enzyme entity in mammalian mitochondria is further addressed in section 5.1. The possibility of SDH becoming more prone to acting as a fumarate reductase due to alternative assembly (Bezawork-Geleta et al., 2018) has not been investigated.

5.1. The risks of using malonate in whole tissues or cells for interrogating SDH directionality

In (Chouchani et al., 2014), Chouchani et al., reported that succinate accumulates in cardiac ischemia as a result of SDH reversal. They deduced that SDH was operating in reverse because i) dimethyl malonate (which is converted to malonate once inside the cell, a competitive inhibitor of SDH) decreased succinate accumulation in the ischemic myocardium and ii) this was suggested by *in silico* flux analysis. The second argument is debatable because their model was overly simplistic, not accounting for participation of metabolites involved in the malate/aspartate shuttle in other reactions, and not including the barrier of an inner mitochondrial membrane. The use of malonate in interrogating SDH directionality in whole tissue carries many risks, some of which have been stressed as early as 1936: Greville (1936) pointed out that at high concentrations (such as 20 mM, used in) malonate inhibits catabolism of acetoacetic acid (Quastel and Wheatley, 1935), inhibits malate dehydrogenase Greville (1936) and increases the formation of ketone bodies Greville (1936). These reports echo a widely known observation of a promiscuous ligand when administered at high doses: off-target effects. Furthermore, one more point to address when using malonate in whole tissues: malonate is transported by the dicarboxylate transporter (DIC), the same transporter that carries succinate (Palmieri et al., 1971); succinate, malate and malonate compete with each other in the kinetics of uptake (Palmieri et al., 1971). Thus, 20 mM malonate will pose a serious challenge for succinate (and malate) transport, especially in light of the fact that malonate is metabolized at a much slower rate (if at all) than succinate. That would shift the reversible reaction catalyzed by succinate-CoA ligase to the right, halting succinate generation. Thus, malonate (and cell-permeable derivatives) are inappropriate for interrogating the role and directionality of SDH in terms of succinate concentration. Finally, there seems to be much more regarding succinate (and malonate) transport that remains to be discovered: based on the fact that the molecules are transported by well-characterized entities out of mitochondria and out of the cell, yet they are considered membrane-impermeable when given exogenously, hence the development of membrane-permeable analogues (Ehinger et al., 2016).

Mindful of the above, it is important to emphasize that the conclusions of Chouchani et al are valid and extremely informative, *i.e.*, succinate does accumulate in ischemia and is a critical driver of reperfusion injury (Chouchani et al., 2014). However, the extent of SDH reversibility to succinate accumulation is rather small; this is important, because as pointed out by Zhang et al (Zhang et al., 2018), the timing of SDH inhibition is critical during ischemia/reperfusion.

6. Mammalian mitochondria do not express a "fumarate reductase" enzyme entity

Fumarate reductases are enzymes present in Gram-negative bacteria, some Gram-positive bacteria, green algae, protozoa, parasitic helminths and some lower marine organisms (freshwater snails, mussels, lugworms, and oysters) but not mammals (Van Hellemond and Tielens, 1994; Van Hellemond et al., 1995). They reduce fumarate to succinate by oxidizing quinols exhibiting a redox potential typically more negative than −60 mV, such as rhodoquinols in eukaryotes and menaquinols or napthoquinols in prokaryotes (Van Hellemond et al., 1995). In my opinion, the very existence of genuine fumarate

reductases attests to the notion that SDH is not reversible to an appreciable extent. However, in *E. coli*, fumarate reductases can work in reverse as SDH (Cecchini et al., 1986; Sucheta et al., 1993), and this is physiologically important when SDH is deleted (Guest, 1981).

The concept of fumarate reductase activity in mammalian mitochondria and especially in cancer cells is pursued by the group of Tomitsuka (Tomitsuka et al., 2009, 2010; Sakai et al., 2012; Tomitsuka et al., 2012). Although the authors acknowledge that fumarate reductase enzyme entities similar to those present in parasites are absent from mammalian cells, they claim that phosphorylation of type II flavoprotein subunit of human mitochondrial SDH increases its fumarate reductase activity (Tomitsuka et al., 2009; Sakai et al., 2013); in turn, they suggest that this leads to sufficient proton pumping through complex I that drives ATP synthesis by the F_o-F_1 ATP synthase. Apart from the fact that they report a fumarate reductase activity in cancer cells on the order of ~0.1% (during normoxia, and double that after hypoxia) of that found in mitochondria of parasites (Sakai et al., 2012), the notion of ATP synthesis by the F_o-F_1 ATP synthase during hypoxia is baseless: the ATP synthase of intact mitochondria requires a membrane potential more negative than ~ −132 mV to synthesize ATP, which is impossible to achieve during ischemia/hypoxia (Chinopoulos, 2011b).

Unfortunately, these reports may have led to the potentially false impression that genuine fumarate reductases reside in mammalian mitochondria, similar to those found in parasites. It is emphasized, however, that Tomitsuka and others do not make the claim of mammalian fumarate reductase enzyme entities. By this mini-review I aim to emphasize that genuine fumarate reductases have never been reported in mammalian tissues, whether healthy or diseased.

7. Conclusions

In tissue ischemia, succinate originates mostly from α-ketoglutarate and upstream metabolites. That also highlights the value of mSLP involved in this process. SDH operating in reverse also exerts a minor contribution in yielding succinate, but only 6–40 times less than that coming from α-ketoglutarate, depending on conditions; for this, suitable quinones with a sufficiently negative redox potential are required for reducing fumarate. The presence of such quinones in mammalian mitochondria remain to be discovered. Finally, although fumarate reductases are very important enzymes in bacterial and lower eukaryotes biochemistry, their existence in mammalian mitochondria remain to be reported.

Funding

The work described in the text was supported by grants FIKP-61822-64888-EATV, VEKOP 2.3.3-15-2016-00012, 2017-2.3.4-TET-RU-2017-00003 and NKFIH KH129567 to C.C.

Acknowledgement

I am indebted to Dr. Gary Cecchini and Dr. Paul S. Brookes for helpful comments and discussions.

References

Ackrell, B.A., Armstrong, F.A., Cochran, B., Sucheta, A., Yu, T., 1993. Classification of fumarate reductases and succinate dehydrogenases based upon their contrasting behaviour in the reduced benzylviologen/fumarate assay. FEBS Lett. 326, 92–94.

Andrienko, T.N., Pasdois, P., Pereira, G.C., Ovens, M.J., Halestrap, A.P., 2017. The role of succinate and ROS in reperfusion injury - A critical appraisal. J. Mol. Cell. Cardiol. 110, 1–14.

Benit, P., Letouze, E., Rak, M., Aubry, L., Burnichon, N., Favier, J., Gimenez-Roqueplo, A.P., Rustin, P., 2014. Unsuspected task for an old team: succinate, fumarate and other Krebs cycle acids in metabolic remodeling. Biochim. Biophys. Acta 1837, 1330–1337.

Bezawork-Geleta, A., Wen, H., Dong, L., Yan, B., Vider, J., Boukalova, S., Krobova, L., Vanova, K., Zobalova, R., Sobol, M., Hozak, P., Novais, S.M., Caisova, V., Abaffy, P., Naraine, R., Pang, Y., Zaw, T., Zhang, P., Sindelka, R., Kubista, M., Zuryn, S., Molloy, M.P., Berridge, M.V., Pacak, K., Rohlena, J., Park, S., Neuzil, J., 2018. Alternative assembly of respiratory complex II connects energy stress to metabolic checkpoints. Nat. Commun. 9, 2221.

Bittl, J.A., Shine, K.I., 1983. Protection of ischemic rabbit myocardium by glutamic acid. Am. J. Physiol. 245, H406–H412.

Bui, D., Ravasz, D., Chinopoulos, C., 2019. The effect of 2-Ketobutyrate on mitochondrial substrate-level phosphorylation. Neurochem. Res.

Cascarano, J., Chick, W.L., Seidman, I., 1968. Anaerobic rat heart: effect of glucose and Krebs cycle metabolites on rate of beating. Proc. Soc. Exp. Biol. Med. 127, 25–30.

Cecchini, G., Ackrell, B.A., Deshler, J.O., Gunsalus, R.P., 1986. Reconstitution of quinone reduction and characterization of Escherichia coli fumarate reductase activity. J. Biol. Chem. 261, 1808–1814.

Chiga, M., Plaut, G.W., 1962. A nucleoside diphosphokinase from liver mitochondria which catalyzes an [32P]ADP-ATP exchange reaction. Biochim. Biophys. Acta 61, 736–740.

Chinopoulos, C., 2011a. The "B Space" of mitochondrial phosphorylation. JNeurosciRes 89, 1897–1904.

Chinopoulos, C., 2011b. Mitochondrial consumption of cytosolic ATP: not so fast. FEBS Lett. 585, 1255–1259.

Chinopoulos, C., 2013. Which way does the citric acid cycle turn during hypoxia? The critical role of alpha-ketoglutarate dehydrogenase complex. J. Neurosci. Res. 91, 1030–1043.

Chinopoulos, C., Seyfried, T.N., 2018. Mitochondrial substrate-level phosphorylation as energy source for glioblastoma: review and hypothesis. ASN Neuro 10, 1759091418818261.

Chinopoulos, C., Gerencser, A.A., Mandi, M., Mathe, K., Torocsik, B., Doczi, J., Turiak, L., Kiss, G., Konrad, C., Vajda, S., Vereczki, V., Oh, R.J., Adam-Vizi, V., 2010. Forward operation of adenine nucleotide translocase during F0F1-ATPase reversal: critical role of matrix substrate-level phosphorylation. FASEB J. 24, 2405–2416.

Chouchani, E.T., Pell, V.R., Gaude, E., Aksentijevic, D., Sundier, S.Y., Robb, E.L., Logan, A., Nadtochiy, S.M., Ord, E.N., Smith, A.C., Eyassu, F., Shirley, R., Hu, C.H., Dare, A.J., James, A.M., Rogatti, S., Hartley, R.C., Eaton, S., Costa, A.S., Brookes, P.S., Davidson, S.M., Duchen, M.R., Saeb-Parsy, K., Shattock, M.J., Robinson, A.J., Work, L.M., Frezza, C., Krieg, T., Murphy, M.P., 2014. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature 515, 431–435.

Colomb, M.G., Cheruy, A., Vignais, P.V., 1969. Nucleoside diphosphokinase from beef heart mitochondria. Purification and properties. Biochemistry 8, 1926–1939.

De Zoeten, L.W., Tipker, J., 1969. Intermediary metabolism of the liver fluke Fasciola hepatica, II. Hydrogen transport and phosphorylation. Hoppe Seylers Z Physiol Chem 350, 691–695.

Ehinger, J.K., Piel, S., Ford, R., Karlsson, M., Sjovall, F., Frostner, E.A., Morota, S., Taylor, R.W., Turnbull, D.M., Cornell, C., Moss, S.J., Metzsch, C., Hansson, M.J., Fliri, H., Elmer, E., 2016. Cell-permeable succinate prodrugs bypass mitochondrial complex I deficiency. Nat. Commun. 7, 12317.

Einbeck, H., 1914. Über das Vorkommen von Bernsteinsäure im Fleischextrakt und im frischen Fleische. Hoppe-Seyler's Zeitschrift für Physiologische Chemie 87, 145–158.

Freminet, A., Leclerc, L., Poyart, C., Huel, C., Gentil, M., 1980. Alanine and succinate accumulation in the perfused rat heart during hypoxia. JPhysiol (Paris) 76, 113–117.

Gailis, L., Benmouyal, E., 1973. Endogenous alanine, glutamate, aspartate, and glutamine in the perfused guinea-pig heart: effect of substrates and cardioactive agents. Can. J. Biochem. 51, 11–20.

Goffeau, A., Pedersen, P.L., Lehninger, A.L., 1967. The kinetics and inhibition of the adenosine diphosphate-adenosine triphosphate exchange catalyzed by purified mitochondrial nucleoside diphosphokinase. J. Biol. Chem. 242, 1845–1853.

Greville, G.D., 1936. Fumarate and tissue respiration: effect of dicarboxylic acids on the oxygen consumption. Biochem. J. 30, 877–887.

Grimolizzi, F., Arranz, L., 2018. Multiple faces of succinate beyond metabolism in blood. Haematologica 103, 1586–1592.

Grivennikova, V.G., Gavrikova, E.V., Timoshin, A.A., Vinogradov, A.D., 1993. Fumarate reductase activity of bovine heart succinate-ubiquinone reductase. New assay system and overall properties of the reaction. Biochim. Biophys. Acta 1140, 282–292.

Gronow, G.H., Cohen, J.J., 1984. Substrate support for renal functions during hypoxia in the perfused rat kidney. Am. J. Physiol. 247, F618–F631.

Guest, J.R., 1981. Partial replacement of succinate dehydrogenase function by phage- and plasmid-specified fumarate reductase in Escherichia coli. J. Gen. Microbiol. 122, 171–179.

Haas, D., 1964. Phosphorylation coupled to the oxidation of Nadh by fumarate in digitonin fragments of beef-heart mitochondria. BiochimBiophysActa 92, 433–439.

Hirst, J., Sucheta, A., Ackrell, B.A.C., Armstrong, F.A., 1996. Electrocatalytic voltammetry of succinate dehydrogenase: direct quantification of the catalytic properties of a complex electron-transport enzyme. J. Am. Chem. Soc. 118, 5031–5038.

Hirst, J., Ackrell, B.A.C., Armstrong, F.A., 1997. Global observation of Hydrogen/Deuterium isotope effects on bidirectional catalytic Electron transport in an enzyme: direct measurement by protein-film voltammetry. J. Am. Chem. Soc. 119, 7434–7439.

Hoberman, H.D., Prosky, L., 1967. Evidence of reduction of fumarate to succinate in perfused rat liver under conditions of reduced O2 tension. Biochim. Biophys. Acta 148, 392–399.

Hochachka, P.W., Owen, T.G., Allen, J.F., Whittow, G.C., 1975. Multiple end products of anaerobiosis in diving vertebrates. Comp. Biochem. Physiol., B 50, 17–22.

Hohl, C., Oestreich, R., Rosen, P., Wiesner, R., Grieshaber, M., 1987. Evidence for succinate production by reduction of fumarate during hypoxia in isolated adult rat heart cells. Arch. Biochem. Biophys. 259, 527–535.

Hunter Jr., F.E., 1949. Anaerobic phosphorylation due to a coupled oxidation-reduction between alpha-ketoglutaric acid and oxalacetic acid. J. Biol. Chem. 177, 361–372.

Jeske, L., Placzek, S., Schomburg, I., Chang, A., Schomburg, D., 2019. BRENDA in 2019: a European ELIXIR core data resource. Nucleic Acids Res. 47, D542–D549.
Johnson, J.D., Mehus, J.G., Tews, K., Milavetz, B.I., Lambeth, D.O., 1998. Genetic evidence for the expression of ATP- and GTP-specific succinyl-CoA synthetases in multicellular eucaryotes. J. Biol. Chem. 273, 27580–27586.
Kacso, G., Ravasz, D., Doczi, J., Nemeth, B., Madgar, O., Saada, A., Ilin, P., Miller, C., Ostergaard, E., Iordanov, I., Adams, D., Vargedo, Z., Araki, M., Araki, K., Nakahara, M., Ito, H., Gal, A., Molnar, M.J., Nagy, Z., Patocs, A., Adam-Vizi, V., Chinopoulos, C., 2016. Two transgenic mouse models for beta-subunit components of succinate-CoA ligase yielding pleiotropic metabolic alterations. Biochem. J. 473, 3463–3485.
Katsura, K., Ekholm, A., Asplund, B., Siesjo, B.K., 1991. Extracellular pH in the brain during ischemia: relationship to the severity of lactic acidosis. J. Cereb. Blood Flow Metab. 11, 597–599.
Manfredi, G., Kiss, G., Konrad, C., Doczi, J., Starkov, A.A., Kawamata, H., Zhang, S.F., Gibson, G.E., Beal, M.F., Adam-Vizi, V., Chinopoulos, C., 2013. The negative impact of alpha-ketoglutarate dehydrogenase complex deficiency on matrix substrate-level phosphorylation. FASEB J. 27, 2392–2406.
Kiss, G., Konrad, C., Pour-Ghaz, I., Mansour, J.J., Nemeth, B., Starkov, A.A., Adam-Vizi, V., Chinopoulos, C., 2014. Mitochondrial diaphorases as NAD(+) donors to segments of the citric acid cycle that support substrate-level phosphorylation yielding ATP during respiratory inhibition. FASEB J. 28, 1682–1697.
Kita, K., Takamiya, S., Furushima, R., Ma, Y.C., Suzuki, H., Ozawa, T., Oya, H., 1988. Electron-transfer complexes of Ascaris suum muscle mitochondria. III. Composition and fumarate reductase activity of complex II. Biochim. Biophys. Acta 935, 130–140.
Kohlhauer, M., Dawkins, S., Costa, A.S.H., Lee, R., Young, T., Pell, V.R., Choudhury, R.P., Banning, A.P., Kharbanda, R.K., Oxford Acute Myocardial Infarction S, Saeb-Parsy, K., Murphy, M.P., Frezza, C., Krieg, T., Channon, K.M., 2018. Metabolomic profiling in acute ST-Segment-Elevation myocardial infarction identifies succinate as an early marker of human ischemia-reperfusion injury. J. Am. Heart Assoc. 7.
Krebs, H.A., 1936. Intermediate metabolism of carbohydrates. Nature 138, 288–289.
Lambeth, D.O., Tews, K.N., Adkins, S., Frohlich, D., Milavetz, B.I., 2004. Expression of two succinyl-CoA synthetases with different nucleotide specificities in mammalian tissues. J. Biol. Chem. 279, 36621–36624.
Laplante, A., Vincent, G., Poirier, M., Des Rosiers, C., 1997. Effects and metabolism of fumarate in the perfused rat heart. A 13C mass isotopomer study. Am. J. Physiol. 272, E74–82.
Maklashina, E., Rajagukguk, S., Iverson, T.M., Cecchini, G., 2018. The unassembled flavoprotein subunits of human and bacterial complex II have impaired catalytic activity and generate only minor amounts of ROS. J. Biol. Chem. 293, 7754–7765.
Massey, V., Singer, T.P., 1957a. Studies on succinic dehydrogenase. III. The fumaric reductase activity of succinic dehydrogenase. J. Biol. Chem. 228, 263–274.
Massey, V., Singer, T.P., 1957b. Studies on succinic dehydrogenase. VI. The reactivity of beef heart succinic dehydrogenase with electron carriers. J. Biol. Chem. 229, 755–762.
Matsuoka, S., Jarmakani, J.M., Young, H.H., Uemura, S., Nakanishi, T., 1986. The effect of glutamate on hypoxic newborn rabbit heart. J. Mol. Cell. Cardiol. 18, 897–906.
Mills, E., O'Neill, L.A., 2014. Succinate: a metabolic signal in inflammation. Trends Cell Biol. 24, 313–320.
Mithieux, G., 2018. Gut nutrient sensing and microbiota function in the control of energy homeostasis. Curr. Opin. Clin. Nutr. Metab. Care 21, 273–276.
Moyle, D.M., 1924. A quantitative study of succinic acid in muscle. I. Biochem J 18, 351–364.
Murphy, M.P., O'Neill, L.A.J., 2018. Krebs cycle reimagined: the emerging roles of succinate and itaconate as signal transducers. Cell 174, 780–784.
Nadtochiy, S.M., Schafer, X., Fu, D., Nehrke, K., Munger, J., Brookes, P.S., 2016. Acidic pH is a metabolic switch for 2-Hydroxyglutarate generation and signaling. J. Biol. Chem. 291, 20188–20197.
Needham, D.M., 1927. A Quantitative Study of Succinic Acid in Muscle. II: The Metabolic Relationships of Succinic, Malic and Fumaric Acids. Biochem. J. 21, 739–750.
Needham, D.M., 1930. A quantitative study of succinic acid in muscle: glutamic and aspartic acids as precursors. Biochem. J. 24, 208–227.
Nemeth, B., Doczi, J., Csete, D., Kacso, G., Ravasz, D., Adams, D., Kiss, G., Nagy, A.M., Horvath, G., Tretter, L., Mocsai, A., Csepanyi-Komi, R., Iordanov, I., Adam-Vizi, V., Chinopoulos, C., 2016. Abolition of mitochondrial substrate-level phosphorylation by itaconic acid produced by LPS-induced Irg1 expression in cells of murine macrophage lineage. FASEB J. 30, 286–300.
Palmieri, F., Prezioso, G., Quagliariello, E., Klingenberg, M., 1971. Kinetic study of the dicarboxylate carrier in rat liver mitochondria. European journal of biochemistry / FEBS 22, 66–74.
Pell, V.R., Chouchani, E.T., Frezza, C., Murphy, M.P., Krieg, T., 2016. Succinate metabolism: a new therapeutic target for myocardial reperfusion injury. Cardiovasc. Res. 111, 134–141.
Penney, D.G., Cascarano, J., 1970. Anaerobic rat heart. Effects of glucose and tricarboxylic acid-cycle metabolites on metabolism and physiological performance. Biochem. J. 118, 221–227.
Pershad, H.R., Hirst, J., Cochran, B., Ackrell, B.A., Armstrong, F.A., 1999. Voltammetric studies of bidirectional catalytic electron transport in Escherichia coli succinate dehydrogenase: comparison with the enzyme from beef heart mitochondria. Biochim. Biophys. Acta 1412, 262–272.
Peuhkurinen, K.J., Takala, T.E., Nuutinen, E.M., Hassinen, I.E., 1983. Tricarboxylic acid cycle metabolites during ischemia in isolated perfused rat heart. Am. J. Physiol. 244, H281–H288.
Pisarenko, O.I., Solomatina, E.S., Studneva, I.M., Ivanov, V.E., Kapelko, V.I., Smirnov, V.N., 1983. Effect of glutamic and aspartic acids on adenine nucleotides, nitrogenous compounds and contractile function during underperfusion of isolated rat heart. J. Mol. Cell. Cardiol. 15, 53–60.
Pisarenko, O.I., Solomatina, E.S., Ivanov, V.E., Studneva, I.M., Kapelko, V.I., Smirnov, V.N., 1985. On the mechanism of enhanced ATP formation in hypoxic myocardium caused by glutamic acid. Basic Res. Cardiol. 80, 126–134.
Pisarenko, O.I., Solomatina, E.S., Studneva, I.M., 1986. The role of amino acid catabolism in the formation of the tricarboxylic acid cycle intermediates and ammonia in anoxic rat heart. Biochim. Biophys. Acta 885, 154–161.
Quastel, J.H., Wheatley, A.H., 1935. Studies in fat metabolism: acetoacetic acid breakdown in the kidney. Biochem. J. 29, 2773–2786.
Ravasz, D., Kacso, G., Fodor, V., Horvath, K., Adam-Vizi, V., Chinopoulos, C., 2018. Reduction of 2-methoxy-1,4-naphtoquinone by mitochondrially-localized Nqo1 yielding NAD(+) supports substrate-level phosphorylation during respiratory inhibition. Biochimica et Biophysica Acta Bioenergetics 1859, 909–924.
Rouslin, W., Broge, C.W., 1989. Regulation of mitochondrial matrix pH and adenosine 5'-triphosphatase activity during ischemia in slow heart-rate hearts. Role of Pi/H+ symport. J. Biol. Chem. 264, 15224–15229.
Sakai, C., Tomitsuka, E., Esumi, H., Harada, S., Kita, K., 2012. Mitochondrial fumarate reductase as a target of chemotherapy: from parasites to cancer cells. Biochim. Biophys. Acta 1820, 643–651.
Sakai, C., Tomitsuka, E., Miyagishi, M., Harada, S., Kita, K., 2013. Type II Fp of human mitochondrial respiratory complex II and its role in adaptation to hypoxia and nutrition-deprived conditions. Mitochondrion 13, 602–609.
Sanadi, D.R., Fluharty, A.L., 1963. On the mechanism of oxidative phosphorylation. VII. The energy-requiring reduction of pyridine nucleotide by succinate and the energy-yielding oxidation of reduced pyridine nucleotide by fumarate. Biochemistry 2, 523–528.
Perille, T., Sanborn, T., Gavin, W., Berkowitz, S., Lesch, M., 1979. Augmented conversion of aspartate and glutamate to succinate during anoxia in rabbit heart. AmJPhysiol 237, H535–H541.
Slater, E.C., 1950. The components of the dihydrocozymase oxidase system. Biochem. J. 46, 484–499.
Sucheta, A., Ackrell, B.A., Cochran, B., Armstrong, F.A., 1992. Diode-like behaviour of a mitochondrial electron-transport enzyme. Nature 356, 361–362.
Sucheta, A., Cammack, R., Weiner, J., Armstrong, F.A., 1993. Reversible electrochemistry of fumarate reductase immobilized on an electrode surface. Direct voltammetric observations of redox centers and their participation in rapid catalytic electron transport. Biochemistry 32, 5455–5465.
Taegtmeyer, H., 1978. Metabolic responses to cardiac hypoxia. Increased production of succinate by rabbit papillary muscles. CircRes 43, 808–815.
Taegtmeyer, H., Peterson, M.B., Ragavan, V.V., Ferguson, A.G., Lesch, M., 1977. De novo alanine synthesis in isolated oxygen-deprived rabbit myocardium. J. Biol. Chem. 252, 5010–5018.
Thunberg, T., 1925. Zur Kenntnis Der Dehydrogenasen der geschlechtszellen. Skandinavisches Archiv Für Physiologie 46, 137–142.
Toenniessen, E.B., 1930. Über den oxidativen Abbau der Kohlenhydrate im Saugertiermuskel, insbesondere über die Bildung von Bernsteinsaure und Brenztraubensaure. Hoppe-Seyler's Zeitschrift für Physiologische Chemie 187, 137–159.
Tomitsuka, E., Kita, K., Esumi, H., 2009. Regulation of succinate-ubiquinone reductase and fumarate reductase activities in human complex II by phosphorylation of its flavoprotein subunit. ProcJpnAcadSerB PhysBiolSci 85, 258–265.
Tomitsuka, E., Kita, K., Esumi, H., 2010. The NADH-fumarate reductase system, a novel mitochondrial energy metabolism, is a new target for anticancer therapy in tumor microenvironments. AnnNYAcadSci 1201, 44–49.
Tomitsuka, E., Kita, K., Esumi, H., 2012. An anticancer agent, pyrvinium pamoate inhibits the NADH-fumarate reductase system–a unique mitochondrial energy metabolism in tumour microenvironments. JBiochem 152, 171–183.
Tretter, L., Patocs, A., Chinopoulos, C., 2016. Succinate, an intermediate in metabolism, signal transduction, ROS, hypoxia, and tumorigenesis. Biochim. Biophys. Acta 1857, 1086–1101.
Urban, P.F., Klingenberg, M., 1969. On the redox potentials of ubiquinone and cytochrome *b* in the respiratory chain. European journal of biochemistry / FEBS 9, 519–525.
Van Hellemond, J.J., Tielens, A.G., 1994. Expression and functional properties of fumarate reductase. Biochem. J. 304 (Pt 2), 321–331.
Van Hellemond, J.J., Klockiewicz, M., Gaasenbeek, C.P., Roos, M.H., Tielens, A.G., 1995. Rhodoquinone and complex II of the electron transport chain in anaerobically functioning eukaryotes. J. Biol. Chem. 270, 31065–31070.
Weil-Malherbe, H., 1936. Studies on brain metabolism: the metabolism of glutamic acid in brain. Biochem. J. 30, 665–676.
Weinberg, J.M., Venkatachalam, M.A., Roeser, N.F., Nissim, I., 2000. Mitochondrial dysfunction during hypoxia/reoxygenation and its correction by anaerobic metabolism of citric acid cycle intermediates. Proc. Natl. Acad. Sci. U.S.A 97, 2826–2831.
Wiesner, R.J., Rosen, P., Grieshaber, M.K., 1988. Pathways of succinate formation and their contribution to improvement of cardiac function in the hypoxic rat heart. Biochem. Med. Metab. Biol. 40, 19–34.
Wilson, M.A., Cascarano, J., 1970. The energy-yielding oxidation of NADH by fumarate in submitochondrial particles of rat tissues. BiochimBiophysActa 216, 54–62.
Zhang, J., Wang, Y.T., Miller, J.H., Day, M.M., Munger, J.C., Brookes, P.S., 2018. Accumulation of succinate in cardiac ischemia primarily occurs via canonical krebs cycle activity. Cell Rep. 23, 2617–2628.