REVIEW

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The metabolism rationale for applying of succinate-based compositions to maintain high performance in a human organism

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Abstract

Design of an effective succinate-based agent for the use in sports has required a profound analysis of the main action mechanisms of the agent in question. Our paper reflects basic principles, which are decisive for the design of the offered succinate-based agent to increase the organism performance and the rate of recovery after intensive physical loading. We have treated a special role of the metabolic conversions of succinate in energy exchange of mithochondria: high energy efficiency, possibility of beneficial oxidation under oxygen deprivation, anaerobic formation and possible consequences of the above phenomenon. The listed key factors have determined the applications of succinate in practice in order to maintain the energy exchange as well as design a number of anti-hypoxia means. It is assumed that the treated peculiarities of the succinate metabolism can provide the basis for formation of a signal, regulatory role of this molecule in the organism environment.

Keywords

Succinate, Metabolism, Succinic acid, Hypoxia

Imprint

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Introduction

For thousands of years there have been created legends and tales of healing properties assigned to amber powder and amber oil [1]. One of the first documented cases of pharmaceutical uses of succinic acid is Hagers Handbuch published in German and Russian since 1856 till 1999 [2, 3]. Known is the St.Peterburg's version "Handbook of Pharmaceutical and Medical & Chemical Practice" dated back to the XIX century. It is interesting that at that time to normalize the human condition, recommended have been succinate-based composition "Mixtura tonico-nervina Stahl".

Participation of succinic acid (SA) in the metabolic processes has been discovered much later: in 1910 by Battelli and Stern [4]. In the 1930th Gozsy B. and Szent-Gyorgyi A. as well as H.A. Krebs in his autonomous research in parallel have identified and established the SA participation role in oxidation & reduction conversion processes in the energy exchange [5, 6] in the tricarboxylic acid (TCA) cycle, which is also known as the Krebs cycle. This discovery has promoted studies and design of pharmaceutical succinate-based compositions (SBC) targeted at the maintenance of the cell energetics under loading conditions. At present, there are succinate-based agents available, which are used in treatment of brain ischemia [7] and blood loss [8]; there are also parapharmaceutical Biologically Active Additives (BAA) on the succinate basis known, which prevent development of meteopathy [9], are beneficial in mitigating menopausal syndrome [10], elevating the human performance [11, 12] and the resistance to alcohol intoxication [13, 14]; the family of these substances includes some veterinary drugs, too [15], etc. Described has been a great variety of exemplary cases of applications of different SBC types in medicine and veterinary [16, 17, 18]. The range of the above SBC products includes well-known Reamberin, Limontar, Mexidol, Yantavit, Mithomin, Enerlit-Klima, Amberen, Potensa, Antip, RU-21, Mithocalcedar etc. The other family of the products designed for elevation of the performance offers Enerlit, YantarIn-Sport, Miodon, Signalom active and Signalom pro Sport. Initially, the idea of the SBC developers has been to use them as a succinate source to maintain the cell energetics. Therefore, we consider first of all the participation role of succinate in metabolic conversions, which are directly linked with intense physical loads.

Succinic acid as an intermediate in the energy exchange

Within the Krebs cycle, succinic acid (SA or succinate) is produced as a result from the oxidative decarboxylation of α-ketoglutarate and the progress of the succinate tyokinase reaction. The next step is when SA (succinate) is oxidized to fumarate by succinate dehydrogenase (SDH), which is not only a ferment in the TCA cycle, but also complex II in the respiratory chain of the mithochondria. The papers by Chance B. [19], Kondrashova M.N. [20, 21, 22] and some other researchers have demonstrated that there is a uniquely high power output produced by mithochondria due to succinate oxidation. The process of the succinate oxidation outperforms all intermediates found in the energy exchange for the oxygen consumption rates and the ATP synthesis, value of the transmembrane electrochemical potential of hydrogen ions $\Delta\mu H^+$, generated on the inner membrane of mithochondria, as well as for the capability to maintain energy-dependent processes like reverse electron transfer (RET) or accumulation of the Ca^{2+} ions. The succinate oxidation results in a release per time unit of much greater energy equivalents than it is the case with the oxidation of any other substrate in the TCA cycle or any fat acids in the β-oxidation reactions.

M.N.Kondrashova and her Scientific School have presented their own concept of a special role of the succinate oxidation in mithochondria in energy supply required for the functioning cycle "rest – performance – recovery" [20, 21, 22]. This concept has played a leading role in the proper understanding of the fact that a high energy power due to the succinate oxidation is a prerequisite for a success in the use of SBC under high energy consumption conditions, or energy deficit and acidosis, under adaptation to heavy loads and post-loading recovery [23].

Anti-hypoxia effect produced by succinate-based compositions

The most vivid example to illustrate the succinic oxidation and formation features can be found under hypoxia. Acute hypoxia ranging up to anoxia is attributed to most functional loading cases and should be considered to be at the root of many adaptive and pathological states. It should be remembered that even under normoxia there can be detected some hypoxia-affected areas, which may appear due to heterogeneity in oxygen supply of different areas in tissues, cells and mithochondria [24, 25]. Tissue heterogeneity in the pO2 distribution can be explained by different lengths of the diffusion path used to transport oxygen to the cells located at different distances from the respective blood vessels. And in addition, it is well known that at rest not all of the capillaries are involved in the operation. Therefore, the farthest cells, which are located at the greatest distances from the arterioles and the artery part of the capillary net, are affected by hypoxia. The same is applicable to the mithochondria located at the greatest distances from the cell surface. Under a considerable surge in the tissue functional activity, there is mismanagement or discord between a relatively slow and/or deficient mobilization of the blood circulation system plus oxygen transport, on the one hand, and a very fast transition of the cells and tissues from their rest to their activity, on the other hand. The most pronounced disagreements in the energy demands can be established between the cells being at rest and those being active in the excitable tissues, which are found in our heart, skeletal muscles and of course our nervous system. Energy expenditures required by the excitable tissues may rapidly grow by a factor of ten and over. As a consequence, the amount of the tissue pO2 decreases, the number of hypoxia-affected areas rises and some temporarily available zones of anoxia appear. In our further considerations, we dwell on differences in conversions of SA in the TCA cycle under the hypoxia and anoxia or anaerobiosis conditions.

Due to high affinity of citochrome oxidase for oxygen, the transport of the reductive equivalents and the oxidative phosphorylation in the respiratory chain is maintained even under deep hypoxia. Lowering oxygen concentrations up to 04,-0,7 μM does not stop functioning of complexes II, III and IV [26, 27]. But it has been revealed that the redox state of respiratory carriers and cytochrome oxidase in tissues are more sensitive to a decrease in pO2 [27, 28] as it is the case in vitro. In particular, for the first 5 seconds under heavy hypoxia (with an oxygen concentration at a level of 20 μM), in isolated tissue sections, pyridine nucleotides are much greater reduced than the other transporters in the respiratory chain [28]. The same differences have been reported for a perfused organ during the transition from normoxia to anoxia [29]: in the hypoxic transition state, practically full reduction of pyridine nucleotides has been observed with a sufficiently high degree of the oxidization of flavopro-

teides. As a rule, under the hypoxic conditions, the oxidation of the NAD-dependent substrates is disrupted, the NADH/NAD ratio significantly grows, and some preconditions for the prioritized oxidation of succinate are generated [30]. It has been detected that complex I is highly sensitive to actions of a great variety of damaging factors and inhibitors, represented by different lipophylic compounds [31, 32]. Besides, it has been established that complex I can lose its prosthetic flavine mononucleotide group [33,34]. Due to an effect of increased concentrations of the nitrogen monoxide and other nitrolyzing compounds, formed in the cell under oxygen deficiency conditions, complex I leaves its active state A for its inhibited state D [35]. Barbiturates, acetaldehyde and rotenone reproduce this situation and make it possible to simulate it in vitro with the total inhibition of complex I and consumption of oxygen in the oxidation of NAD-dependent substrates, for example, β-oxybutirate (see Figure 1 herein). It has turned out that of great importance are the presence of electronophylic metabolites like oxaloacetate and the progress of the fumarate reductase reaction that promotes the succinate formation by the reductive conversion in the Krebs cycle. Owing to functioning of complexes II, III and IV [26, 27, 36], succinate produced due to a high level of NADH is immediately oxidized (see Figure 1A herein). Malonate as the SDH inhibitor stops both the succinate oxidation and the fumarate reductase reaction. According to recorded data on the malonate-sensitive oxygen consumption in the presence of rotenone and by generation of transmembrane potential ΔΨ (see Figure1 B herein), we can estimate dynamically the contribution of the NAD-dependent substrates, for instance of α-ketoglutarate or some mixtures of substrates like α-ketoglutarate with aspartate, or malate with pyruvate etc., to the succinate formation.

The prioritized oxidation of succinate under hypoxia (against the background of a high degree of the NADH reduction) is provided by the availability of the oxidized flavoproteides and coenzyme Q and a flow of the reductive equivalents at the terminal portion of the respiratory chain. It is interesting that even under normoxia (really under hyperoxia in the incubation cuvette) in state 4 according to B. Chance and G.R. Williams [36], due to an increase in the degree of the NADH reduction, observed is the prevailed oxidation of succinate that is recorded by loss of radioactivity of a radioactive tracer in vitro in the intact rabbit's heart

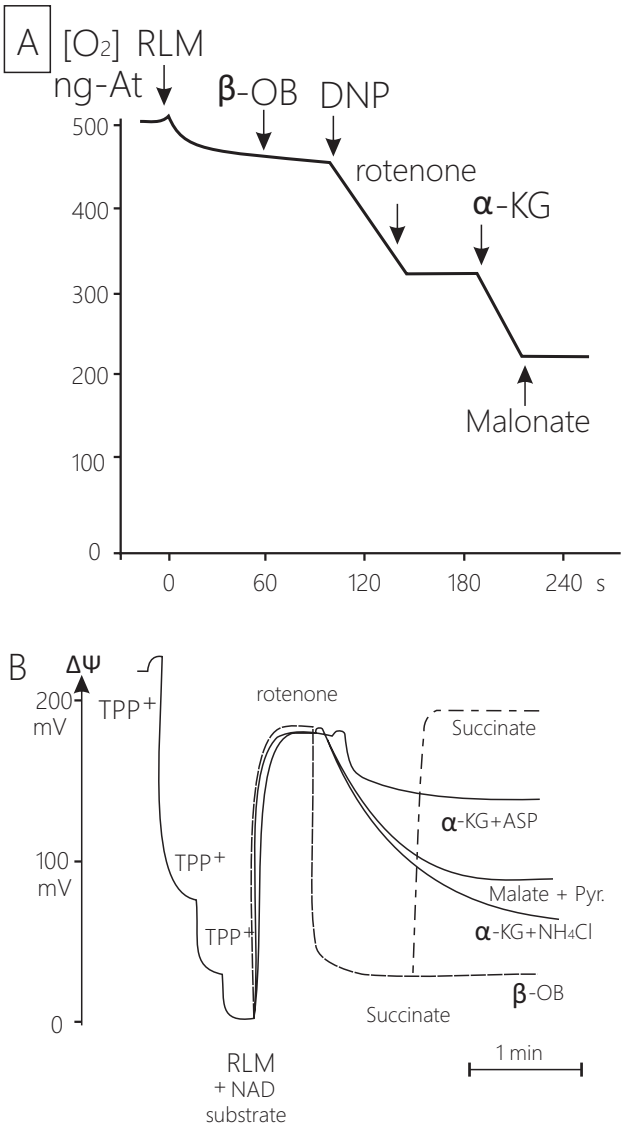

Figure 1. Addition of rotenone into suspension of respirating mithochondria suppresses oxygen consumption (A) in the oxidation of β-oxybutirate (β-OB). By adding α-ketoglutarate (α-KG) we can easily restore the respiration of mithochondria in the liver (RLM under uncoupling of the oxidative phosphorylation by 2-4-dinitrophenol (DNP). Generation of the transmembrane potential takes place despite the fact that there is rotenone block (B) in the presence of α-ketoglutarate (α-KG) with aspartate (ASP), malate with pyruvate or α-ketoglutarate with ammonia. The proper full-scale transmembrane potential is generated under the oxidation of the added succinate. The incubating medium has been composed as follows: 250 mM sucrose, 10 mM tris-HCl (pH 7,4), 10 mM KCl, 3 mM MgCl2, and 3 mM KH2PO4. Concentration of mithochondria is 3 mg per ml; t 26°C. All substrates have been added with a final concentration of 5 mM. DNP – 30 μmol, rotenone -10 μmol. Oxygen consumption data have been recorded with polarography. The transmembrane potential has been measured with the use of the selective electrode according to changes in concentrations of the lipophyl cation of tetraphynilphosphonium (TPP+).

mithochondria [37]. In state 4, a high value of the ATP/ADP ratio for the mechanism of the respiratory control retards the flow of the reductive equivalents that results in an increase of the NADH/NAD+ratio. During the oxidation of the traced pyruvate in mithochondria, a non-proportional drop of the tracer concentration in succinate (contrary to the theoretical stoichiometry of TCA) in state 4 is detected. Conversely, in state 3, when values of both ratios NADH/NAD+ and ATP/ADP sharply decrease, a non-proportional accumulation of the tracer in succinate appears, while it lowers in the intensively oxidizing NAD-dependent substrates [37].

The priority of succinate to be oxidized in vivo under the hypoxia conditions can be evidenced by almost doubled drop of the succinate concentration in the liver in rats placed in an altitude chamber, when simulating the true altitude conditions of 8000 m [23]. It has been demonstrated by N.A.Glotov that upon staying "at the above elevation" for 2 hours, a significant reduction in the succinate concentrations in blood, the liver, the heart and the kidney in the rats has been reported, while a doubled concentration of the NAD-dependent substrates has been detected [38]. An indirect argument in favor of the prioritized oxidation of succinate under an arbitrary, short-time, for 40 seconds, breath holding, is an abnormal decrease in the value of the respiratory coefficient $R=\Delta CO_2/\Delta O_2$ up to 0,45÷0,55 in the first portion of the exhaled air [39]. Under eupnea, the R value in volunteer test subjects has been reported to reach 0,95÷0,97. We think the observed decrease in the R value reflects the oxidation of those substrates, which have not been subjected to decarboxylation (in the absence of anywhere pronounced respiratory acidosis). In this connection, first and foremost succinate can be classed with the above type of the substrates. The oxidation of lipids is accompanied by a decrease in the R value to 0,7. It should be noted that with developing respiratory acidosis the R value can exceed 1,0 due to an increase of pCO_2.

Some researchers believe that it is precisely the possibility to retain the oxidation of succinate that favors the maintenance of the oxidative phosphoryliation under hypoxia [8, 23, 28, 30, 36, 37,]. So, we can summarize it as indicated in Figure 3 herein: inhibition of the oxidation of the NAD-dependent substrates at the level of complex I and the prioritized oxidation of succinate under hypoxia.

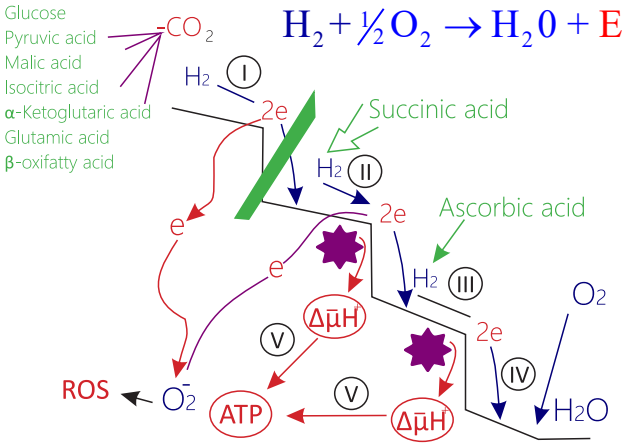

Figure 2. Against the background of the hypoxic inhibition of the oxidation of the NAD-dependent substrates [26, 27, 35], the oxidation of succinate is further maintained. SDH delivers a pair of electrons into the respiratory chain irrespective of the degree of the NADH reduction and functioning of complex I. It has been shown that the energy released during the transfer of the pair of electrons (2e) through the respiratory chain to oxygen is converted into transmembrane elechtrochemical potential $\Delta\mu H^+$. With the use of complex V – ATP-synthase, $\Delta\mu H^+$ provides for phosphorylation of ADP to ATP. Despite a decrease in the ATP/O value, a high rate of the succinate oxidation under the maintenance of functioning of complexes II, III, IV and V makes possible to keep a sufficiently high energy efficiency of the oxidative phosphorylation. It has been also shown a single electron leakage promoting generation of oxygen superoxide ó2 as a progenitor of other reactive oxygen species (ROS).

The presented materials suggest that under the hypoxia conditions, the functional disruption of the link between the NAD-dependent dehydrogenases in the TCA cycle and the respiratory chain and the selective prioritizing of the succinate oxidation change essentially the progress of the redox reactions in the TCA cycle. So, under the oxygen deprivation succinate maintains energy production in mithochondria.

Anaerobic formation of succinate in mithochondria

Under the anaerobic conditions, in the suspension of isolated mithochondria, similar to an organ with an interrupted blood supply, just upon expiration of several seconds, a ten- to fifty-fold accumulation of succinate can be easily found [40-47]. Hochachka P.W. and G.N. Somero have described a spike of endogenous succinate concentration at the level of the organism as a whole in deep-sea animals and divers [48]. Usually the TCA cycle reductive conversion of oxaloacetate is assumed to be a source of succinate.

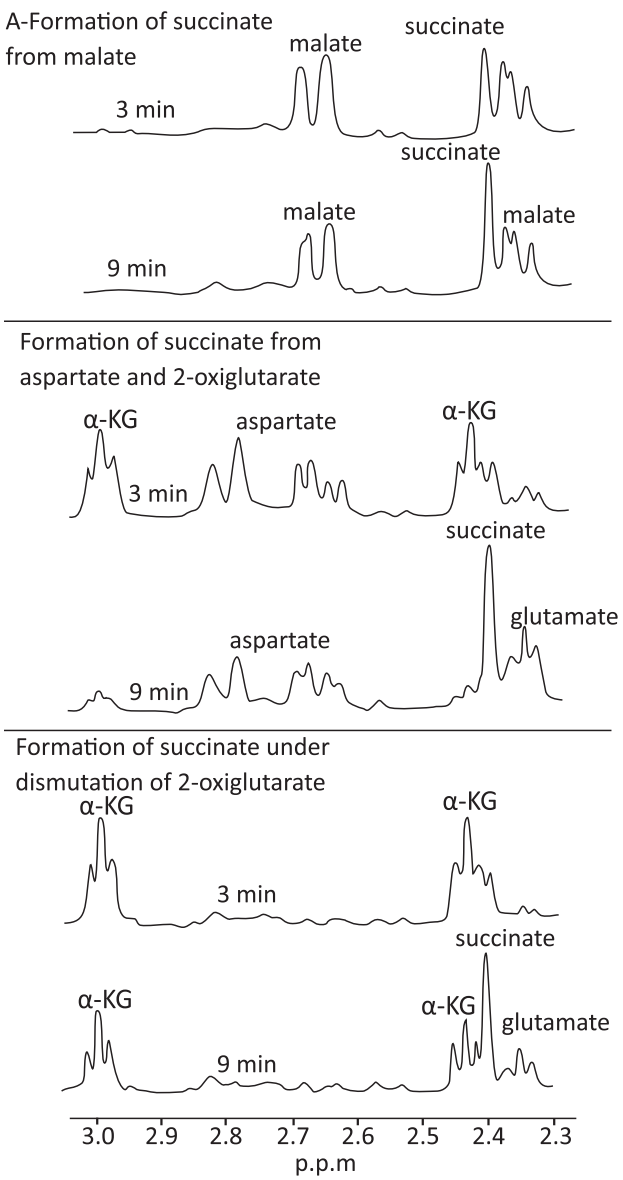

Figure 3. The H1-NMR spectra under mithochondria incubation (16 mg of protein per ml) in the rat liver with different substrates under the closed respiratory chain at the level of complex III with antimycin A (0,35 μg/mg mithochondrial protein). Cuvette volume is 0.5 ml, t 26°C. Incubating medium composition: 100 mM KCl, 3 mM KH2PO4, 3mM MgCl2, 0,5mM EGTA, 0,4 mM ADP, 2 0 mM tris-HCl buffer (pH 7,4) and 2,5% D2O. Substrates(A – 5 mM malate, B – 5 mM aspartate and 5 mM α-ketoglutarate, C – 5 mM α-ketoglutarate and 2,5 mM NH4Cl. Each curve is a result of 90 accumulations for 90 seconds. There are curves upon 3 and 9 minutes of mithochondria incubation. (Operated by M.S.Okon with NMR-Spectrometer [50]).

Table 1
Changes in ratios between the pathways of anaerobic formation of succinate in mithochondria in the rat's heart as percentages of [succinate] growth in consecutive time intervals

Pathway of anaerobic formation of succinate	Duration of anaerobiosis			
	3 min.	4,5 min.	6 min.	7,5 min.
A. Reductive conversion in TCA	100%	39%	–	–
B. Coupled oxidation of α-ketoglutarate	–	51%	–	–
C. Anaerobic dismutation of α-ketoglutarate	–	11%	100%	100%

We have measured with the use of H1 Nuclear Magnetic Resonance (NMR) spectroscopy in accordance with the NMR technique by O.I.Pissarenko [40] theoretically possible pathways of accumulation of succinate under the stopped respiratory chain in the mithochondria in the heart, the renal cortex and the brain in rats and guinea pigs [23]. As it is evident from data given in Figure 3 herein, we can identify in the examined mithochondria at least three metabolic pathways of the anaerobic formation of succinate (AFS).

The most known AFS pathway is the reductive conversion in the TCA cycle from oxaloacetate (OAA) or malate (see Figure 4 A). The most powerful AFS pathway is represented by coupled fluxes, when the reductive conversion in the TCA cycle supports the oxidative part of the TCA cycle (see Figure 4 B herein). And, finally, the anaerobic dismutation of α-ketoglutarate according to Krebs-Kohen [49] in the presence of excess of ammonia (of the order of 1-1,5 mM) takes place (see Figure 4 C). In this case, under intense loading, deamination of adenyle nucleotides due to energy deficit is the prime contributor.

We have demonstrated in the schemes given in Figure 5 the anaerobic pathways of succinate formation in that step sequence (A, B, C), which is implemented in mithochondria without regard to from what tissue they have been separated. Initially, against the background of the preserved oxidative phosphorylation, process A has taken place. A driving force for this is a high degree of the reduction of NADH and the oxidative phosphorylation due to an increased level in ADP and non-organic phosphate. The next step is oxidation: process B is started due to the appearance of NAD+. As endogenous ammonia is de-energized and accumulated, process C is initiated: it's the anaerobic dismutation of α-ketoglutarate. By the example of mithochondria in the heart as indicated in Table 1 herein, a typical contribution of these pathways as a percentage of the AFS value in consecutive time intervals under the anaerobic incubation of mithochondria.

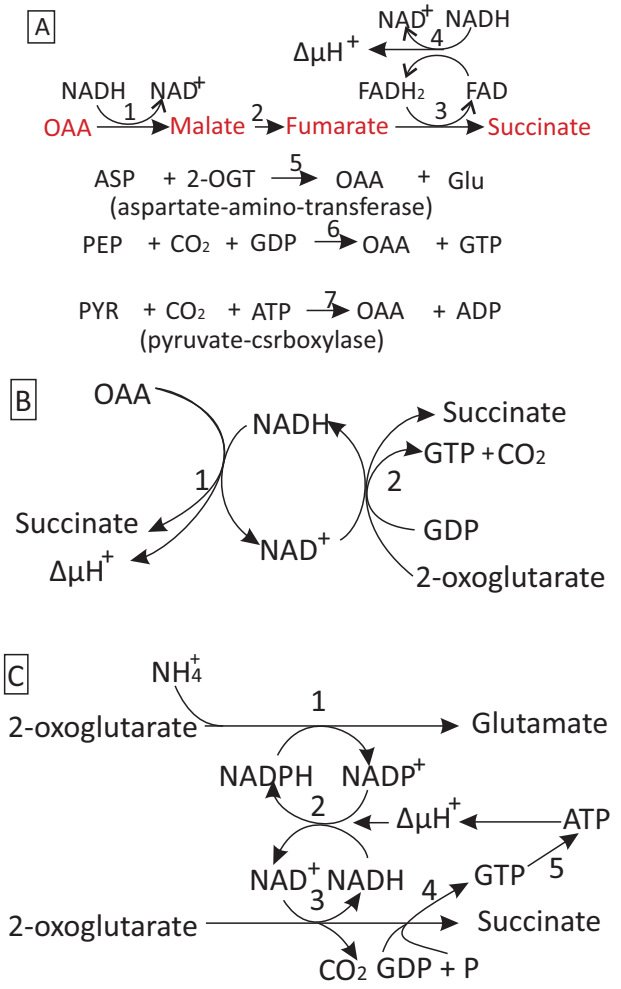

Figure 4. Scheme of anaerobic formation of succinate in mithochondria in different organs. The reductive conversion in the TCA cycle due to excess of NADH from oxaloacetate (OAA) to succinate (Figure 4 A) takes place under participation of malate dehydrogenase (1), fumarase (2) and SDH undertaking the role of fumarate reductase (3). In the fumarate reductase reaction oxidized are the reduced flavoproteid and Coenzyme-Q. This is responsible for the fact that the reductive equivalents are transferred from complex I to the oxidized Coenzyme-Q, and the oxidative phosphorylation of ATP (4) [51- 55] occurs. The OAA source may be aspartate (ASP) in the aspartate aminotransferase reaction (5), phosphoenolpyruvate (PEP) in the phosphoenolpyruvate carboxinase reaction (6) and pyruvate (PYR) in the pyruvate carboxilase reaction (7). Reaction (6) in rats is presented to 95% in the cytosol, while that in pigeons, guinea pigs, rabbits and human individuals appears practically equally in the mithochondria and cytosol. Under the stress conditions, activity of the cytosol phosphoenolpyruvate carboxinase significantly rises: hormonal induction of synthesis de novo takes place. Figure 4 B (coupling of two AFS flows): the reductive conversion in the TCA cycle from OAA to succinate (1) favors the oxidation of NADH to NAD+, which is reduced in the usual progress of the oxidative reactions in the TCA cycle, among them in the progress of the oxidation of isocitrate and α-ketoglutarate to succinate (2). In this case, the oxidative phosphorylation occurs in the same manner as it is the case with situation A in the course of the reductive conversion in the TCA cycle. At the same time, the substrate phosphorylation of GTP at the level of succinyl-CoA, formed as a result from the oxidative decarboxylation of α-ketoglutarate, takes place. Under the anaerobic dismutation of α-ketoglutarate (see Figure 4 C) [49], in the glutamate dehydrogenase reaction (1) NAD(P)H is oxidized due to the reductive amination of one molecule of α-ketoglutarate to glutamate. The oxidized NADP+ is reduced by transhydrogenase at the expense of NADH (2). For this purpose, required is generation of $\Delta\mu H^+$ of the order of 100 mV that is twice less than required for the ATP phosphorylation. The NAD+ oxidized by transhydrogenase and reductive amination favors the oxidative decarboxylation of another, the second, molecule of α-ketoglutarate (3) to succinyl-CoA, which provides for the substrate phosphorylation (4) of GTP. Nucleoside diphosphate kinase (5) discharges a small pool of GTP by transfer of phosphate to ADP: ATP is formed. It is conceivable that it is just ATP that is used to maintain $\Delta\mu H^+$ required for the transhydrogenase reaction (2).

So, under the anaerobic conditions in the TCA cycle, succinate is formed and accumulated similar to the case when lactate is stored in the anaerobic glycolysis.

It is of importance that, as opposed to anoxia, under hypoxia, when we deal with heterogeneity in the pO_2 distribution, both AFS and the succinate oxidation may take place at the same time, in parallel, in different areas. Where the anaerobic conditions are available, SDH operates as fumarate reductase, with reducing fumarate to succinate. In those areas, where higher pO_2 values are found, the terminal portion of the respiratory chain is in operation, and SDH functions as succinate: coenzyme-Q-oxidoreductase. In doing so, a fumarate-succinate shuttle is provided. First the fumarate-succinate shuttle has been detected between the lungs and peripheral tissues under hypoxic exposure in animals [54]. We suppose this sort of shuttles takes place between the mithochondria in cells and between the cells within the same tissue type due to heterogeneity in the pO_2 level in different areas and in the degree of the reduction of the respiration-related carriers.

Consequences of anaerobic formation of succinate (AFS)

Originally AFS in mithochondria have been considered as an exclusively adaptive process, which favors formation of energy-rich compounds and maintenance of the functional condition of poly-ferment systems in mithochondria in the absence of oxygen. It has been suggested that an additional bonus in this case is a fast way to tackle an energy deficit under the re-oxygenation due to the oxidation of the accumulated succinate. Of course, the energy-yielding role of AFS in mithochondria is small and cannot meet even the basic requirements of mithochodria in the TCA cycle with the stopped respiratory chain (see Figure 5 A herein). The energy output from AFS may only cover the requirement to avoid a sudden drop in the ATP/ADP ratio for 3-6 minutes under the blockade of adenylate translocase by carboxy-atractyloside and ATPase in mithochondria by oligomycine (see Figure 5 B herein) [50].

It is probable that AFS in cells under the anaerobic conditions is a minor energy source, which is available in addition to glycolysis [23]. So, under the cold cardioplegia conditions, with suppression of the energy consumers in the intentionally arrested heart, when the blood supply is interrupted, the beneficial contribution of AFS to the intactness of the myocardium is most pronounced [44-46].

Reperfusion damage and oxidation of the accumulated succinate

A large body of research papers shows that a post-ischemic oxidation spike of succinate accumulated in the heart is combined with an explosive acceleration in formation of reactive oxygen species (ROS), which are responsible for development of post-ischemic reperfusion damages [56-62]. An intensive formation of ROS at the moment of reperfusion is determined by a rapid pO_2 growth. It is favorable to free-radicals'-single-electron leakages with the reduced carriers – the formation of ROS, which increases in proportion to the pO_2 value in a wide range of the oxygen concentrations, even in the transition from normoxia to hyperoxia. It has been demonstrated that the process of generation of ROS in mithochondria can be provided in full only in the case, when the $\Delta\mu H^+$ value exceeds 150 mV [58-60]. However, it has turned

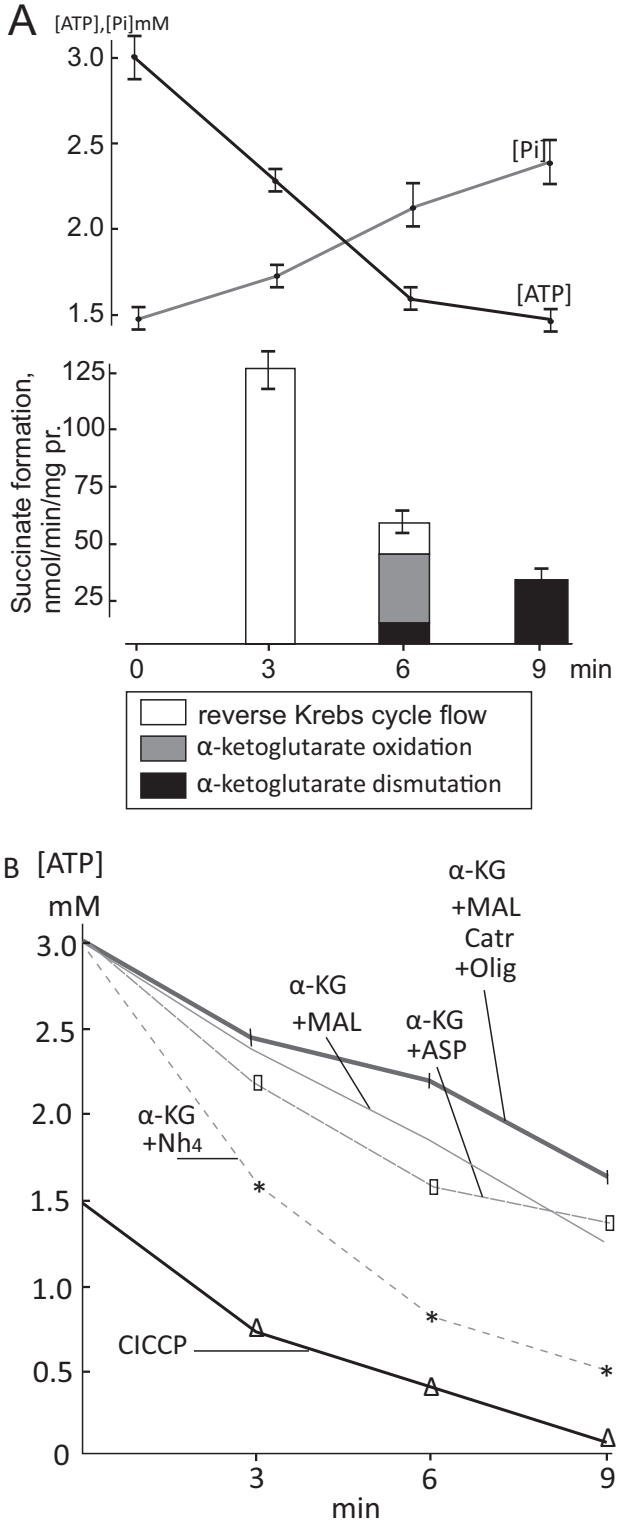

Figure 5. Changes in the energy condition of mithochondria in the rat's liver under AFS. Legend: A: The [ATP] and [Pi] dynamics in the presence of α-ketoglutarate and aspartate with the traced change in the AFS pathways. ATP and phosphate concentrations are estimated according to the respective 31P-NMR spectra. B: The [ATP] dynamics in the mithochondria suspension under incubation in the presence of different substrate sources of AFS and two inhibitors at the same time: 10-5M carboxy-atractyloside (inhibitor of adenylate translocase) and 10-5M oligomycine (inhibitor of H+ATPase). The incubation conditions are the same as shown in Figure 3 above herein..

out that the pO2 level and the $\Delta\mu H^+$ value or the electrical $\Delta\Psi$ thereof and the ΔpH component are not the leading factors in the ROS generation. The key role plays a high degree of the reduction of the respiration carriers in complexes I and III, which are maintained in the presence of oxygen due to a high ATP/ADP ratio in state 4 according to Chance B.- Williams G.R.

Hence it follows that in case of the oxidation of succinate, which is accumulated in excess after ischemia, the situation with generation of ROS is not so simple as it is reported in many papers including those mentioned by us [57,58]. There is a lot of research works, which demonstrate a direct relationship between the excess generation of ROS and the oxidation of the succinate excess and active operation of ADH (succinate dehydrogenase, succinate: ubichinon oxidoreductase) with the reduction during the reverse transfer of electrons (RTE) of complex I as well as a rapid growth of the degree of the reduction of components in complexes II and III. However there is little likelihood thereof [62], for at least one reason: at the moment of reperfusion, plenty of ATP consumers reproduce active state 3, i.e. an excess of ADP and even uncoupling of the oxidative phosphorylation. In the circumstances, neither maintenance of a high ratio NAD(P)H/NAD(P)+ or keeping of a great value of $\Delta\mu H^+$ is possible, since de-energization and destroy of intactness of the membranes in mithochondria take place.

In the above mentioned experiments on AFS we have estimated the phosphoryl potential (the ATP/ADP ratio and the pool of adenyl nucleotides) and the maintenance of respiration control (in our experiment it has been shown as dependence of the AFS rate on the ATP/ADP value in isolated mithochondria). It has been evidenced that even in vitro under relatively comfortable conditions (the closed system), namely free of calcium overloading, without external ATP consumers, in the presence of excessive substrates, at a temperature decreased to 26°C, de-energization of mithochondria and uncoupling of the oxidative phosphorylation inevitably take place that is growing for 9 minutes of the incubation with the stopped respiratory chain [23]. This situation has been treated in detail in experimental works and reviews [60, 61, 62, 63]. In the cells, a heat-associated ischemia, reperfusions against the background of not isolated energy consumers, post-ischemic calcium overloading and opening of the mitochondrial permeability transition pore (PTP) hinder maintaining $\Delta\mu H^+$, a high NADH/NAD+ ratio and RTE. In addition, there is a pool of papers, which have demonstrated that there is protection of mithochondria from peroxidation of lipids (POL) of the membranes at the expense of the succinate oxidation [64]. Conversely, inhibition of SDH provokes enhancing of the pro-oxidant activity of mithochondria [65] and increases formation of superoxide radicals followed by developing apoptosis [66]. In vitro exogenous succinate hinders inactivation of SDH in case of initiation of lipid peroxidation by entry of Fe2+ [67, 68, 69] and inhibits the lipid peroxidation induced by the Fe2+-adenylate complex or potentiated by ageing of mithochondria [70]. It should be noticed that the oxidation of succinate is more resistant to damaging actions by pro-oxidants than the oxidation of the NAD-dependent substrates, in particular of α-ketoglutarate (KG) and pyruvate [54]. It follows that in order to identify in vivo specific conditions, under which oxidation of the succinate excess may produce pro- or anti-oxidant effects, required are further special investigations.

Our materials presented herein treat some metabolism-related grounds for the application of succinate and succinate-based compositions in order to maintain the energy exchange, especially under hypoxia, as well as an anti-oxidant means. However our analysis of the role and effects produced by succinate cannot cover all the aspects. The offered metabolic interpretation is based on studies on actions and effects made by sufficiently high concentrations of succinate in vitro and high dosages of succinate in vivo, which are comparable to those millimolar concentrations, which are capable of producing a direct effect through their participation in the metabolic processes. There are not so many physiology grounds for the interpretation of the above matters, at least due to the fact that succinate delivered via the the stomach and the gastro-intestinal system is intensively used by the epithelium of the stomach and the bowel, the microbiome, the liver etc. As a result, only very small quantities of exogenous succinate can reach mithochondria in other tissues in the organism. Studies conducted for last decades have considerably extended the conceptual scopes of possible succinate application effects on the human organism. In this connection, we should mention that discovered and widely studied is the role of exomithochondrial succinate as stabilizer of the cytosol transcriptional adaptational hypoxia-inducing factor HIF1α [71]. It has been established that extracellular succinate is a specific ligand of the cell succinate

receptor SUCNR1 [72], which is called by some researchers a stress-receptor. The special role played by succinate in metabolism of mithochondria has been justified and preserved by evolution at the regulatory level in the systems of higher hierarchical levels. This scientific field requires further particular treatment and a thorough analysis.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

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