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Influence of substrate activation (hydrolysis of ATP by first steps of glycolysis and β-oxidation) on the effect of enzyme deficiencies, inhibitors, substrate shortage and energy demand on oxidative phosphorylation

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Abstract

In intact tissues respiratory substrates (glucose, fatty acids) must be activated with the use of ATP before they may be oxidised and used for energy (ATP) production. This activation by product constitutes an example of a typical positive feedback. In the present paper, the influence of substrate activation on the effect of inborn enzyme deficiencies, inhibitors, lowered oxygen tension, respiratory fuel shortage and increased energy demand on respiration and ATP synthesis is studied with the aid of the dynamic computer model of oxidative phosphorylation in isolated mitochondria developed previously. Computer simulations demonstrate that, in the case where oxidative phosphorylation in the whole organism is partially inhibited, the necessity of substrate activation can have significant impact on the relationship between the activity of (particular steps of) oxidative phosphorylation (or the value of energy demand) and the respiration rate. Depending on the sensitivity of ATP usage to ATP concentration, substrate activation may either slightly enhance the effect of the decrease in the oxidative phosphorylation activity (increase in energy demand) or may lead to a non-stability and sudden collapse of the respiration rate and phosphorylation potential below (above) a certain threshold value of oxidative phosphorylation activity (energy demand). This theoretical finding suggests a possible causal relationship between the affinity of ATP usage to [ATP] and the tissue specificity of mitochondrial diseases.

Keywords: Oxidative phosphorylation; Mitochondria; Respiratory substrate; Mitochondrial diseases; Computer model

1. Introduction

Oxidative phosphorylation is the main source of energy in the form of ATP in most animal tissues under most conditions. The response of this process to different factors decreasing its activity, for instance inborn enzyme deficiencies, physiological inhibitors (e.g. NO), external poisons (CO, KCN, heavy metals, alcohol, chloroform), diminished oxygen pressure or respiratory substrate shortage is very important for understanding and predicting the effect of these factors on the functioning of the cell under normal and pathological conditions.

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The dependence of the respiration rate on the activity of particular oxidative phosphorylation complexes was studied in isolated mitochondria by means of specific inhibitor titration [1,2]. A threshold value of the enzyme activity was evidenced, above which the respiration rate was essentially independent on the enzyme activity and below which oxygen consumption decreased steeply with the decrease in enzyme activity. These experimental studies were supplemented with computer simulations, which also predicted the existence of the threshold [3–5]. This threshold behaviour appeared to be universal for all the oxidative phosphorylation complexes in mitochondria isolated from different tissues working in various conditions (different energy demands and oxygen concentrations), although the threshold value was different in different cases [1–7]. The threshold curves were interpreted within the paradigm of the metabolic control analysis [2].

The studies in the isolated mitochondria system were undoubtedly very important for general understanding of the effect of different factors decreasing the activity of oxidative phosphorylation on the rate of respiration and ATP synthesis. Nevertheless, several quantitative kinetic properties of ATP production in isolated mitochondria titrated with specific inhibitors cannot be directly extrapolated to partially inhibited mitochondria working in intact tissues. In the case of inborn deficiencies of (subunits of) enzymes encoded in mitochondrial DNA it has been demonstrated that random segregation of mtDNA molecules to daughter mitochondria during mitochondria divisions, leading to origination of two pure mitochondria lines: one wild-type and one mutated (the so-called 'binary mitochondria heteroplasmy'), enhances significantly the deleterious effect of mutations [5]. Namely, the same degree of a decrease in a given complex activity/concentration causes a much greater decrease in the respiration rate in the case of the binary mitochondria heteroplasmy than in the case of specific inhibitor titration of such a complex in isolated mitochondria [5]. In the binary mitochondria heteroplasmy the degree of inactivation of the entire mitochondrial oxidative phosphorylation is equal to the degree of deficiency of a given enzyme and thus deficiencies in all oxidative phosphorylation complexes have the same effect on oxidative phosphorylation.

Additionally, only the activity of (an artificial system of) ATP usage is directly activated in the isolated mitochondria system during an increased energy demand, while a parallel activation of ATP usage and of (different steps of) ATP supply is likely to take place during transition from resting state to active state in intact tissues [8–11]. This parallel activation in the ATP supply-demand system significantly lessens the effect of enzyme deficiencies, inhibitors, diminished oxygen pressure and substrate shortage on oxidative phosphorylation [12].

Finally, one of the most fundamental characteristics of living organisms is that they are nonlinear systems. In the energetic aspect this property manifests itself as the fact that some energy is necessary in order to produce more energy. In the particular case of the bioenergetic system of the animal cell some minimal amount (concentration) of ATP is needed for the activation of main respiratory substrates: glucose and fatty acids, before these substrates may be oxidised in glycolysis, β-oxidation and/or Krebs cycle, donate electrons on the respiratory chain and ultimately drive ATP synthesis in mitochondria. The reactions catalysed by hexokinase and phosphofructokinase in glycolysis and by acyl-CoA synthetase in fatty acid β-oxidation, constituting the first stages of these metabolic pathways, use ATP as substrate (and hydrolyze this compound to ADP or AMP). Therefore, at low ATP concentrations (high [ATP] inhibits substrate dehydrogenation) there takes place an activation of the substrate dehydrogenation system by its product, which leads to a typical positive feedback. The necessity of substrate activation in intact tissues is inevitable for purely thermodynamic reasons. On the other hand, the isolated mitochondria system is normally devoid of substrate activation, because isolated mitochondria are provided with already 'activated' respiratory substrates representing intermediate metabolites of Krebs cycle or glycolysis, such as succinate, 2-oxoglutarate or pyruvate. Therefore, the dependence between the respiration rate and the activity of (particular complexes of) oxidative phosphorylation measured in isolated mitochondria

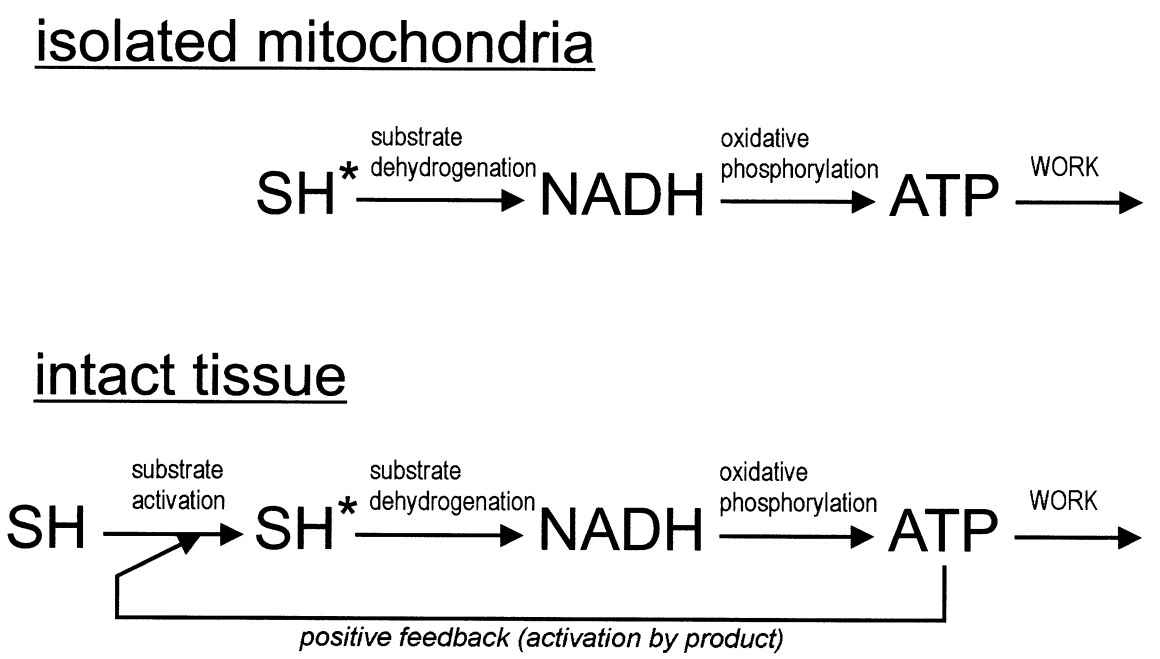

Fig. 1. Schematic comparison of the oxidative phosphorylation system without substrate activation by ATP (isolated mitochondria) and with substrate activation by ATP (intact tissues). In intact tissues there takes place a positive feedback—activation by product. SH, respiratory substrate (glucose, fatty acid); SH*, 'activated respiratory substrate' = intermediate metabolite being a product of the activation of a respiratory substrate with the use of ATP (e.g. fructose 1,6-bis-P, pyruvate or acyl-CoA; of course, fructose 1,6-bis-P is absent in isolated mitochondria).

does not take into account the possible effect of substrate activation, which is present in intact tissues. Fig. 1 presents schematically the discussed difference between the isolated mitochondria system, which lacks substrate activation, and intact tissues, where substrate activation is present. This scheme does not involve glycolytic ATP production because, as it is discussed below, anaerobic glycolysis cannot produce ATP for a long time when oxidative phosphorylation in all tissues in an organism is inhibited—the accumulating lactate and progressing acidification block glycolysis.

The present article investigates in the theoretical way, with the aid of the computer model of oxidative phosphorylation developed previously [3,9], the possible influence of the substrate activation on the effect of enzyme deficiencies, inhibitors, decreased oxygen concentration, substrate shortage and increased energy demand on the respiration rate. In particular, the case is considered where oxidative phosphorylation in all tissues is inhibited and therefore lactate produced in some tissues (e.g. muscle) cannot be effectively oxidised in other tissues. It is demonstrated that substrate activation may lead, especially in the case of high affinity of ATP usage to [ATP], to non-stability of the system, expressed as a sudden collapse of the respiration rate and phosphorylation potential below a certain threshold value of the activity of (a given complex of) oxidative phosphorylation or above a certain threshold value of energy demand. It is suggested that different affinities of ATP usage to [ATP] in different tissues may contribute to the tissue specificity of mitochondrial diseases. Finally, it is demonstrated that parallel activation in the ATP supply-demand system during an increased energy demand can protect the system against the harmful effect of the necessity of substrate activation on oxidative phosphorylation.

2. Theoretical procedures

In this study, oxidative phosphorylation is modelled in the same way as in [3]. The following enzymes/processes/metabolic blocks are taken into account explicitly within the model: substrate dehydrogenation (hydrogen supply to the respiratory chain comprising glycolysis, fatty acid β-oxidation, Krebs cycle and so on), complex I, complex III, complex IV (cytochrome *c* oxidase), proton leak, ATP synthase, ATP/ADP carrier, phosphate carrier, adenylate kinase, ATP-usage system. The rate of each reaction/process is described by an appropriate kinetic equation. The time variations of the metabolite concentrations that constitute independent variables (NADH, ubiquinol, cytochrome *c*, O_2, internal protons, internal ATP, internal P_i, external ATP, external ADP and external P_i), are expressed in the form of a set of ordinary differential equations. The other (dependent) variable values (other metabolite concentrations, thermodynamic forces and so on) are calculated from the independent variable values. The set of differential equations is integrated numerically. In each iteration step, new values of rates, concentrations and other parameters are calculated on the basis of the corresponding values from the previous step. The Gear procedure was used for numerical integration and the simulation programs were written in the FORTRAN programming language. A more detailed description of the model is given elsewhere [3]. The complete description is accessible on the web site: http://www.mol.uj.edu.pl/~benio/.

In the present article two types of the kinetic description of substrate dehydrogenation were used: one without and one with substrate activation. Substrate dehydrogenation without substrate activation was described as in the original version of the model [3]:

$$v_{DH} = k_{DH} \frac{1}{\left(1 + \frac{K_{mN}}{NAD^+/NADH}\right)^{p_D}} \quad (1)$$

where $K_{mN} = 100$, $p_D = 0.8$. Substrate dehydrogenation with substrate activation was described as follows:

$$v_{DH} = k_{DH} \frac{1}{\left(1 + \frac{K_{mN}}{NAD^+/NADH}\right)^{p_D}} \cdot \frac{1}{\left(1 + \frac{K_{mATP}}{ATP}\right)} \quad (2)$$

where $K_{mATP} = 200$ μM, which corresponds approximately to the value of the apparent Michaelis–Menten constant of hexokinase and phosphofructokinase for [ATP] [13]. The following kinetic description of ATP usage was used:

$$v_{UT} = k_{UT} \frac{1}{1 + \frac{K_{mA}}{ATP}} \quad (3)$$

where $K_{mA} = 150$ or 15 μM.

Three different cases were modelled

Case 1: Substrate dehydrogenation without substrate activation Eq. (1), ATP usage relatively sensitive to [ATP] ($K_{mA} = 150$ μM); this mode corresponds to the isolated mitochondria system with hexokinase + glucose as an artificial ATP-using system. The solid line in Figs. 2–7 represents this case.

Case 2: Substrate dehydrogenation with substrate activation Eq. (2), ATP usage relatively sensitive to [ATP] ($K_{mA} = 150$ μM); this mode corresponds to intact tissues with ATP usage of relatively low affinity to [ATP]. This case is represented by the dotted line in Figs. 2–7.

Case 3: Substrate dehydrogenation with substrate activation (Eq. (2)), ATP usage relatively insensitive to [ATP] ($K_{mA} = 15$ μM); this mode corresponds to intact tissues with ATP usage of relatively high affinity to [ATP]. The dashed line in Figs. 2–7 represents this case.

In the simulations presented in Figs. 2–5 energy demand (rate constant of ATP usage k_{UT}) was fixed to give the respiration rate at normal (not inhibited) activity of oxidative phosphorylation equal to 54% of the respiration rate in state 3 (the maximal respiration rate in isolated mitochondria). In the simulations presented in Fig. 6 different levels of relative energy demand (expressed as a

multiplicity of the resting energy demand in intact muscle [14]) were fixed as indicated on the *x*-axis of Fig. 6. In the simulations presented in Fig. 7 an *n*-fold increase in energy demand in relation to resting energy demand (standardised for 1) was accompanied by an $n^{0.5}$-fold activation of the ATP-producing system (substrate dehydrogenation and all steps of oxidative phosphorylation but proton leak) (the parallel-activation mechanism).

Different relative activities of oxidative phosphorylation (*x*-axis) in the simulations presented in Fig. 2 were fixed by a gradual decrease, in subsequent simulations, of the relative rate constants of all oxidative phosphorylation steps but substrate dehydrogenation from 1 (normal activity) to 0. Similarly, different relative activities of complex III of the respiratory chain (*x*-axis) in the simulations presented in Fig. 3 were fixed by a gradual decrease, in subsequent simulations, of the relative rate constant of this complex from 1 (normal activity) to 0. The dependence of the respiration rate on oxygen concentration (Fig. 4) was obtained by fixing different constant oxygen concentrations between 3 and 0 μM. Because the concentration of respiratory substrates is not taken into account explicitly within the computer model used, the effect of respiratory substrate shortage (Fig. 5) was rendered by a decrease in the relative effective activity (rate constant) of the substrate dehydrogenation block to the extent indicated on the *x*-axis of Fig. 5.

3. Theoretical results

Fig. 2a, presents the simulated dependence of the respiration rate on the relative activity (concentration) of mitochondria in Case 1, Case 2 and Case 3. In Case 1 (system without substrate activation, corresponding to the isolated mitochondria system) the dependence has the form of a typical threshold curve, evidenced previously both in theoretical and experimental way [5]—the respiration rate changes little above some threshold value of mitochondria activity (indicated as threshold I in Fig. 2a) and diminishes steeply below this value (equal to approx. 0.5 in Fig. 2a). In Case 2 (system with substrate activation, corresponding to intact tissues with ATP usage relatively sensitive to [ATP]) a very similar dependence takes place as in Case 1: only one threshold (threshold I) is present and the threshold value of relative mitochondria activity is equal to approximately 0.5. On the other hand, a significantly different dependence between the respiration rate and the relative mitochondria activity takes place in Case 3 (system with substrate activation, corresponding to intact tissues with ATP usage relatively insensitive to [ATP]). Here, the respiration rate is even less dependent on mitochondria activity above the threshold I. However, below the threshold the oxygen consumption decreases much more steeply than in Case 1 and Case 2. Additionally, there appears a second threshold (indicated as threshold II in Fig. 2a) (at relative mitochondria activity of approximately 0.27), below which a sudden collapse of the respiration rate (and phosphorylation potential) takes place. This threshold is caused by the positive feedback present in the system, as shown in Fig. 1-below some critical ATP concentration there is too little ATP to activate the respiratory substrates and to start substrate dehydrogenation. As a result, a non-stability appears, in which respiration and [ATP] fall suddenly to zero. Generally, two thresholds are present in Case 3. They will be consequently called in the present paper the 'threshold I' (the 'normal' threshold which is present also in the system without substrate activation) and 'threshold II' (the 'non-linearity' threshold caused by the positive feedback in the system with substrate activation).

Apparently, the difference between the kinetic background of Case 2 and of Case 3 seems to be rather small, because the affinity of ATP usage to [ATP] in Case 3 is only by one order of magnitude greater than in Case 2. For this reason, it may seem surprising that the dependence of the respiration rate on relative mitochondria activity is so different in Case 2 and Case 3. In fact, this dependence in Case 2 is very similar to the dependence in Case 1, where no substrate activation is present. The above observation testifies to a very high sensitivity of the behaviour of the system to the K_m constant of ATP usage for [ATP] (K_{mA}). This fact may have important consequences for tissue specificity of mitochondrial diseases (see below).

Since the rates of both ATP usage and substrate dehydrogenation are described within the model as dependent on [ATP], the ATP concentration is a very important variable in the considered system. Fig. 2b presents the simulated dependence of [ATP] on the concentration/activity of mitochondria. It can be seen that the level of ATP starts to decrease significantly below the threshold I. At threshold II ATP suddenly drops from approximately 10 μM to zero. This can be hardly seen in Fig. 2b because of the scale of the *y*-axis (relatively high [ATP] at normal mitochondria activity). The collapse of the energetic state is much more evident if the phosphorylation potential is considered instead of [ATP] (not shown).

It must be stressed that the threshold value of both threshold I and threshold II strongly depends on energy demand—the greater the energy demand, the higher the threshold values (theoretical results not shown). As it was mentioned in Theoretical Procedures, in the simulations presented in Fig. 2 the energy demand (the rate constant of ATP usage k_{UT}) was fixed to give the respiration rate equal to approximately 50% of the state 3 respiration rate (maximal respiration rate in isolated mitochondria).

It is assumed in the simulations presented in Fig. 2 that all mitochondrial complexes are inhibited by a certain factor to the same extent, which is equivalent to complete inhibition of all complexes in some fraction of mitochondria and thus to switching off these mitochondria completely. This assumption seems to be strictly valid in the case of binary heteroplasmy of mtDNA mutations [5]. However, most factors (nuclear DNA mutations, inhibitors, poisons) affect selectively only some mitochondrial enzymes; additionally, inactivated complexes are here homogeneously distributed among mitochondria. Nevertheless, if the oxidative phosphorylation system is treated as a black box (one is not interested how oxidative phosphorylation is inhibited), inactivation of any of the mitochondrial complexes leads to the inactivation of the entire oxidative phosphorylation system (in this case, of course, the overall inhibition of oxidative phosphorylation will be smaller than the degree of inhibition of its single step). Therefore, a large inhibition of one complex is equivalent, from the formal point of view, to a smaller inactivation of entire oxidative phosphorylation (the degree of this inactivation will depend on the flux control coefficient of a given enzyme over the ATP synthesis flux).

Fig. 3, presents the simulated dependence of the respiration rate on the activity of one mitochondrial complex taken as an example, namely complex III of the respiratory chain, in Case 1, Case 2 and Case 3. The general qualitative pattern is similar here to the pattern shown in Fig. 2. Case 1 and Case 2 evidence one threshold, while two thresholds are present in Case 3. However, it can be clearly seen that the threshold values are shifted here towards lower values of the relative activity of complex III (threshold I appears at approx. 0.15, while threshold II at approx. 0.04 of the normal activity of complex III). Nevertheless, as in the previous case, the threshold values depend strongly on energy demand (not shown). The dependence of the respiration rate on the relative complex activity is generally similar for other mitochondrial complexes (data not shown), although, of course, the threshold values are slightly different for different complexes (however, these threshold values are always significantly lower than threshold values for whole mitochondria presented in Fig. 2a).

The dependence of [ATP] on the activity of complex III is generally similar to the dependence presented in Fig. 2b (not shown): ATP concentration drops significantly below threshold I and suddenly falls to zero at threshold II.

Fig. 4 presents the simulated dependence of the respiration rate on oxygen concentration in Case 1, Case 2 and Case 3. It can be seen that the apparent $K_{0.5}$ constant of oxidative phosphorylation for oxygen is equal to approximately 1 μM in all three cases. Again, Cases 1 and 2 are very similar to each other and exhibit no non-stability. On the other hand, in Case 3 there takes place a sudden collapse of the respiration rate and phosphorylation potential at oxygen concentration of approximately 0.6 μM. The reason is of course the same as above—a positive feedback manifesting itself when ATP concentration falls below some critical value. The chain of events is as follows: decrease in $[O_2] \rightarrow$ decrease in [ATP] $\rightarrow$ decrease in sub-

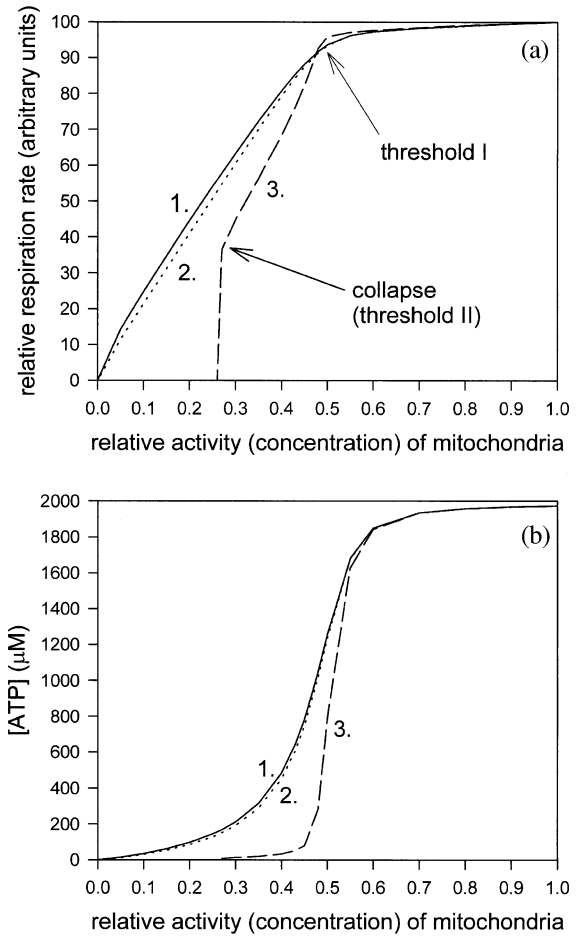

Fig. 2. Simulated dependence of the respiration rate (a) and ATP concentration (b) on the activity of oxidative phosphorylation in mitochondria for Case 1 (no substrate activation, ATP usage relatively sensitive to [ATP]) (solid line), Case 2 (substrate activation present, ATP usage relatively sensitive to [ATP]) (dotted line) and Case 3 (substrate activation present, ATP usage little sensitive to [ATP]) (dashed line).

strate activation → decrease in ATP production → further decrease in [ATP]... and so on. Again, the threshold value of oxygen concentration is positively correlated with relative energy demand (the higher the energy demand, the higher the threshold value). And again, the dependence of [ATP] on oxygen concentration is qualitatively similar to the dependence presented in Fig. 2b (not shown).

The computer model of oxidative phosphorylation used in the present study does not involve explicitly the concentration of respiratory substrates. However, from the formal point of view substrate shortage is equivalent to a decrease in the (apparent) rate constant of substrate dehydrogenation k_{DH} in Eq. (1) or Eq. (2). Such a decrease in the rate constant was used to imitate the effect of substrate shortage in the three cases distinguished in the present article. Fig. 5 presents the simulated dependence of the respiration rate on the relative activity of substrate dehydrogenation in Case 1, Case 2 and Case 3. Here, unlike in the previous simulations, Case 2 is very similar to Case 3, while Case 1 is clearly different. In Case 1 one can observe a typical threshold curve with only one threshold—threshold I. In Case 2 and Case 3 threshold I and threshold II fuse (or, in fact, threshold II is present at a higher value of the activity of substrate dehydrogenation than threshold I). In all three cases [ATP] decreases below the single threshold observed. However, while in Case 1 this decrease is quick but not instant, in Case 2 and Case 3 [ATP] suddenly drops to zero (not shown).

Fig. 6a presents the simulated dependence of the respiration rate on the relative energy demand for the negative-feedback mechanism in Case 1, Case 2 and Case 3. Different levels of energy demand were fixed using different values of the rate constant of ATP usage k_{UT}. The value of energy demand in resting muscle cell, where proton leak accounts for approximately 50% of oxygen consumption [15], was standardised for 1 (this value gives respiration rate equal to approx. 20% of state 3 respiration rate). Zero energy demand corresponds to state 4 and energy demands greater than 1 correspond to active states, in which ATP turnover is greater in the resting state. One can see that up to the relative energy demand of 25, the respiration rate increases in a near-linear manner with energy demand in all three cases. Above this point the behaviour of the system is different in different cases. In Case 1 the respiration rate simply stabilises at some constant value (corresponding to state 3 respiration in isolated mitochondria) when energy demand further increases. Case 2 is similar to Case 1 with the exception that here the respiration rate slightly decreases at higher energy demands. In Case 3 the linear part of the

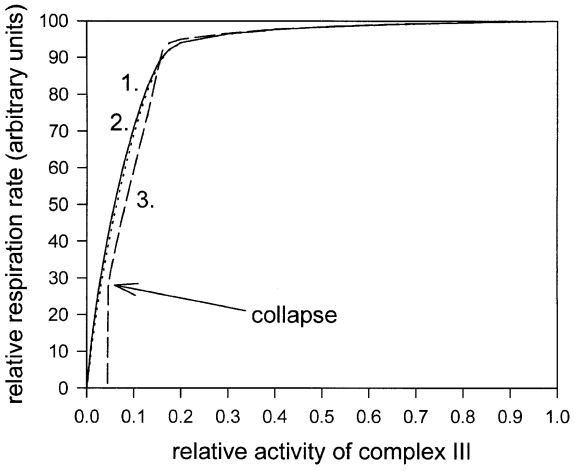

Fig. 3. Simulated dependence of the respiration rate on the activity of complex III of the respiratory chain for Case 1 (no substrate activation, ATP usage relatively sensitive to [ATP]) (solid line), Case 2 (substrate activation present, ATP usage relatively sensitive to [ATP]) (dotted line) and Case 3 (substrate activation present, ATP usage little sensitive to [ATP]) (dashed line).

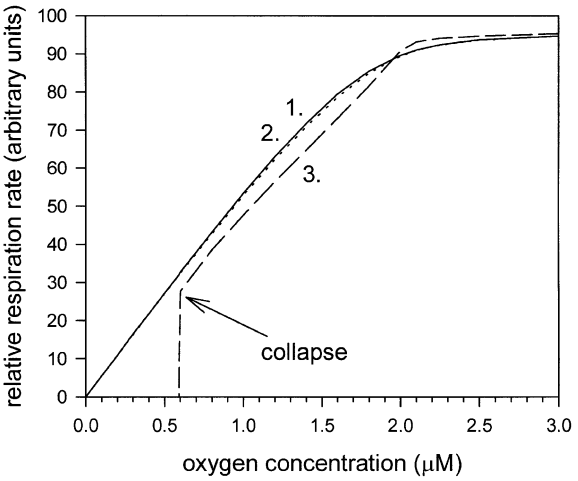

Fig. 4. Simulated dependence of the respiration rate on oxygen concentration for Case 1 (no substrate activation, ATP usage relatively sensitive to [ATP]) (solid line), Case 2 (substrate activation present, ATP usage relatively sensitive to [ATP]) (dotted line) and Case 3 (substrate activation present, ATP usage little sensitive to [ATP]) (dashed line).

discussed dependence is even longer than in the other cases. However, after some critical point (relative energy demand = 26) the respiration rate starts to fall and at relative energy demand of 28 a collapse of oxygen consumption and of phosphorylation potential takes place because of the positive feedback present in the system. Therefore, exceeding some maximal energy demand in Case 3 may lead to an energetic catastrophe in a cell, when the system is stimulated by the output-activation mechanism. This critical increase in energy demand is quite small, corresponding to an approximately 8-fold increase in the respiration rate in relation to resting state, while at least 20–50-fold increase in oxygen consumption is observed in intact skeletal muscle during transition from resting state to intensive exercise [16]. Fig. 6b shows that, below threshold I, [ATP] decreases significantly but continuously in Case 1 and Case 2, while in Case 3 this decrease is more steep, and [ATP] suddenly drops from 45 μM to zero at energy demand of 27. The last event is associated with a collapse in the phosphorylation potential (not shown).

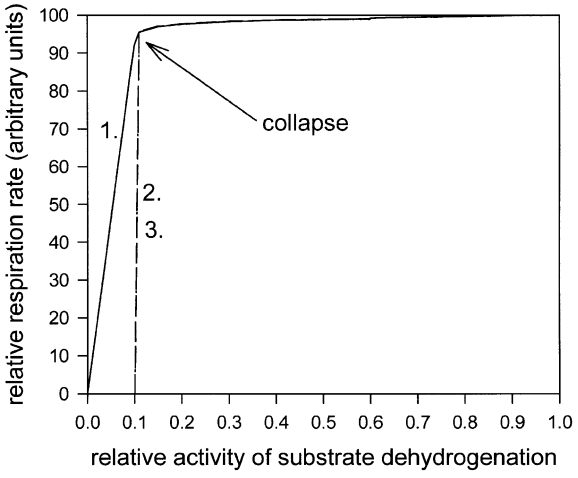

Fig. 5. Simulated dependence of the respiration rate on the apparent activity of substrate dehydrogenation (dependent on respiratory substrate availability) for Case 1 (no substrate activation, ATP usage relatively sensitive to [ATP]) (solid line), Case 2 (substrate activation present, ATP usage relatively sensitive to [ATP]) (dotted line) and Case 3 (substrate activation present, ATP usage little sensitive to [ATP]) (dashed line).

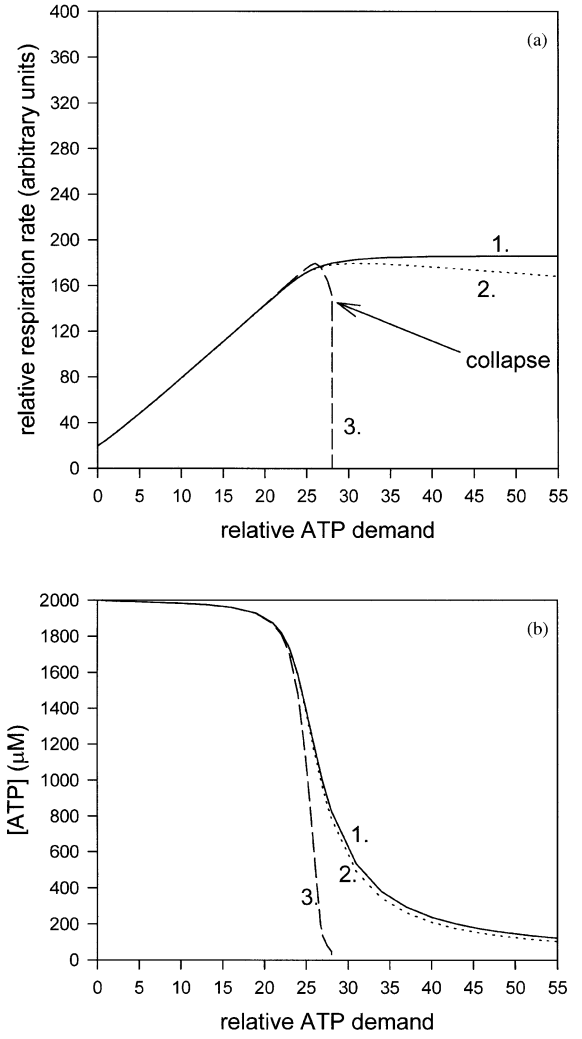

Fig. 6. Simulated dependence of the respiration rate (a) and ATP concentration (b) on the relative energy demand in the case of the negative-feedback mechanism for Case 1 (no substrate activation, ATP usage relatively sensitive to [ATP]) (solid line), Case 2 (substrate activation present, ATP usage relatively sensitive to [ATP]) (dotted line) and Case 3 (substrate activation present, ATP usage little sensitive to [ATP]) (dashed line). Energy demand n (the rate constant of ATP usage) is standardised for 1 in resting state in intact muscle.

Such a catastrophe can be easily prevented when not only ATP usage, but also ATP supply is directly activated by some factor during transition from resting state to intensive exercise (parallel-activation mechanism). Fig. 7 presents the simulated dependence of the respiration rate on relative energy demand in the case of the parallel-activation mechanism in Case 1, Case 2 and Case 3. In these simulations ATP supply was activated $n^{0.5}$ times in parallel with an n-fold activation of ATP usage (energy demand) in relation to resting state. The dependence is very simple here and is identical for all three cases: the respiration rate increases linearly with energy demand in the whole range of energy demand tested. Therefore, the relative increase in the respiration rate and ATP turnover may be much greater here than in the previous case. Additionally, there is no danger of a collapse of oxygen consumption and phosphorylation potential. The ATP concentration was essentially constant for all three cases in the simulations presented in Fig. 7 (not shown).

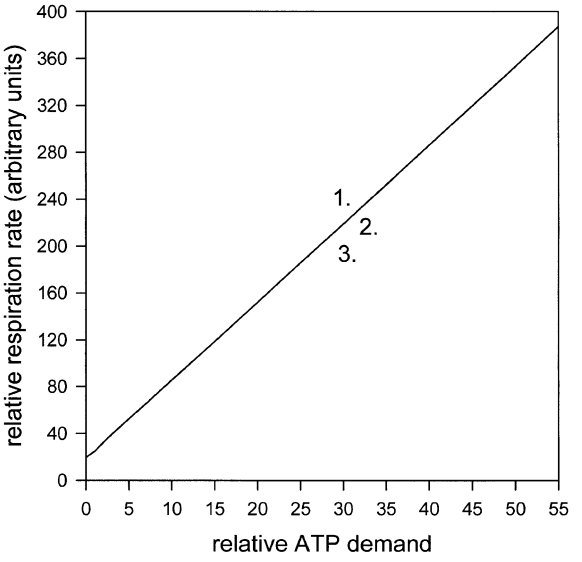

Fig. 7. Simulated dependence of the respiration rate on the relative energy demand in the case of the parallel-activation mechanism for Case 1 (no substrate activation, ATP usage relatively sensitive to [ATP]) (solid line), Case 2 (substrate activation present, ATP usage relatively sensitive to [ATP]) (dotted line) and Case 3 (substrate activation present, ATP usage little sensitive to [ATP]) (dashed line). Energy demand n (the rate constant of ATP usage) is standardised for 1 in resting state in intact muscle. ATP supply is activated $n^{0.5}$ times in relation to resting state, in parallel with an n-fold activation of ATP usage.

In the simulations presented in Figs. 2–6, the threshold ATP concentration, below which the collapse of the respiration rate and phosphorylation potential appeared, was equal to 8–55 μM, depending on conditions (compare Fig. 2b and Fig. 6b and the relevant discussion in the text).

4. Discussion

One of the most fundamental properties of living organisms is that they are non-linear, autocatalytic systems. Their only evolutionary 'purpose', imposed by the natural selection, is to survive, grow and reproduce. Living organisms intake from the surroundings mater and energy, and transform them into the substance (specific organic compounds) and energy of their bodies. The autocatalysis consists here in the fact that in order to sustain biochemical and physiological processes, increase body mass and produce descendants, living beings must already have at disposal some appropriately-organised substance (their bodies) and some energy in a proper form. Appropriate substance and energy are necessary to ensure the continuity of life (in other words, complex living organisms do not originate nowadays spontaneously from inanimate matter and they die if the amount of their substance and/or energy falls below some threshold value).

In the context of animal cell bioenergetics this general rule manifests itself as the thermodynamic need of hydrolysis of some amount of ATP for substrate activation in order to produce much more ATP in the process of oxidative phosphorylation. The activation of the substrate dehydrogenation+oxidative phosphorylation system by its product—ATP—constitutes a typical example of a positive feedback that can lead to a non-stability. This fact may have important impact on the kinetic properties of mitochondria in intact tissues, especially under pathological conditions where the activity of oxidative phosphorylation and ATP concentration is lowered in a result of enzyme deficiency, action of poison or severe oxygen/respiratory substrate shortage. A largely increased energy demand (and in fact any factor that can decrease [ATP] below some critical value) may also cause manifestation of the positive feedback and lead to non-stability. This fact was generally discussed by Reich and Sel'kov [17].

The above property of the bioenergetic system of an intact cell was not involved in the previously-developed model of oxidative phosphorylation in isolated mitochondria [3,9], which was used in a number of theoretical studies on the relationship between the activity of (particular complexes of) oxidative phosphorylation and the respiration rate [3–5,12]. Similarly, substrate activation was absent in the isolated mitochondria experimental model (isolated mitochondria were provided with already 'activated' substrates), which was used in studies on the influence of enzyme deficiencies on oxidative phosphorylation and on the genesis of mitochondrial diseases [1,2,6,7]. In both the cited experimental investigations and theoretical studies the curves of the dependence of the respiration rate on the relative activity of (particular complexes of) oxidative phosphorylation evidenced a single threshold. The present article, which analyses the influence of substrate activation on the kinetic behaviour of the oxidative phosphorylation system in the case where all tissues in an organism have partially inhibited oxidative phosphorylation suggests that the situation prevailing in intact tissues may be more complicated.

In particular, theoretical simulations show that, in the case where the affinity of ATP usage to ATP is significantly larger than the affinity of substrate dehydrogenation to ATP (Case 3), a second threshold (threshold II) appears in addition to the threshold (threshold I) which is present already in the system without substrate activation. Figs. 2–5 show the results of simulations that were intended to model the influence of different factors decreasing the activity of oxidative phosphorylation on the respiration rate. The simulations presented in Fig. 2, where the activity (concentration) of whole mitochondria is changed, correspond to the effect of deficiencies of mitochondrial complexes (subunits) encoded in mitochondrial DNA (mtDNA). As it was discussed previously [5], random segregation of a few mtDNA molecules present in each mitochondrium during mitochondria divisions should lead after a few divisions to origination of two pure mitochondria populations: one wild-type and one mutated (the so-called

binary mitochondria heteroplasmy). In such a case, a complete inactivation of some mitochondrial complex in a result of mtDNA mutation will lead to a complete inactivation of some fraction of mitochondria, containing mutated mtDNA molecules, regardless the flux control coefficient of this complex over the respiration (and ATP synthesis) flux [5].

The simulations presented in Fig. 3 are aimed to reflect the influence of deficiencies of enzymes encoded in nuclear DNA (in this case partially inactivated enzyme molecules are homogeneously distributed among different mitochondria) as well as of physiological inhibitors and external poisons, which in most cases affect specifically particular oxidative phosphorylation complexes.

In Fig. 4 and Fig. 5 there is modelled the effect of decreased oxygen concentration and respiratory substrate shortage, respectively.

For all considered factors that decrease the activity of oxidative phosphorylation, substrate activation may lead to a non-stability of the system and to a sudden collapse of the respiration rate and phosphorylation potential.

In Fig. 6 and Fig. 7 there is compared the effect of substrate activation during an increase in energy demand for two possible mechanisms responsible for adjusting the rate of ATP supply to the rate of ATP usage: output-activation mechanism (only ATP usage is activated directly by some external signal, ATP supply is activated only indirectly, through changes in [ADP]) and parallel-activation mechanism (both ATP usage and ATP supply are directly activated in parallel by some external factor(s), while changes in [ADP] constitute only a secondary, fine-tuning signal). It can be seen that a sudden collapse of the respiration rate (and phosphorylation potential) may take place above some threshold value of energy demand in the case of the output-activation mechanism. On the other hand, the parallel-activation mechanism is able to prevent effectively the danger of an energetic catastrophe in a cell and allows a much greater increase in the respiration rate and ATP turnover than the output-activation mechanism does.

Of course, any computer model may offer at best only a simplified and approximate description of complex reality. Therefore, one could address the question how relevant for the physiological conditions prevailing in intact tissues are the simulations presented above.

First, in some situations, especially in skeletal muscle during intensive exercise, anaerobic glycolysis is an important source of ATP. This could potentially prevent decreasing [ATP] below the threshold value, where the non-stability takes place. The lactate produced by anaerobic glycolysis in muscle can be effectively oxidised by other tissues, for instance heart, liver or brain, what prevents excessive acidification of the organism (which could damage cells and block glycolysis). However, this mechanism is effective only for a relatively short time and only because there exist other tissues, which oxidise lactate and thus counteract the acidification.

On the other hand, enzyme deficiencies and inhibitors, causing long-term, persistent decrease in the activity of oxidative phosphorylation, affect more or less homogeneously all the tissues, and therefore, in this case, some tissues would not be able to oxidise lactate effectively and thus compensate the acidification caused by other tissues.

Second, glucose and fatty acids, although very important, are not the only respiratory substrates. Different tissues may oxidise ketone bodies, lactate/pyruvate or aminoacids. However, ketone bodies, pyruvate and lactate originate mostly either from acetyl-CoA, a product of glycolysis and fatty acid β-oxidation (ketone bodies), or as a product of glycolysis (pyruvate/lactate). Therefore, they constitute already 'activated' intermediate metabolites—the activation of glucose and fatty acids by ATP is needed for their production. As to aminoacids, their oxidation seems to be in most the cases only a minor source of ATP and may lead to poisoning of the organism with ammonium.

Third, in many cell cultures in vitro (especially in the case of tumour cells) cells are able to rely only on glycolytic ATP supply and therefore they do not need oxidative phosphorylation for growth and divisions (they can grow in the absence of oxygen). However, in this case the external medium of a large relative volume can accumulate

much lactate and effectively buffer the acidification brought about by anaerobic glycolysis. Anyway, cultured cells must be transferred after some time to a new medium, not contaminated with lactate and H^+ ions. Therefore, cell cultures do not reflect well the conditions prevailing in intact tissues, where the relative volume of the extracellular space is small and it cannot be replaced after some time with fresh 'medium'.

There exists a great variety of mitochondrial diseases, of which some are lethal (severe diseases) and some are sublethal (mild diseases). In the case of sublethal mitochondrial diseases, some cells die, while other cells are less severely affected. The mild and severe effect of inborn enzyme deficiencies may be potentially correlated with the two thresholds seen in the simulations presented in Fig. 2 and Fig. 3. The threshold I could be responsible for the genesis of mild diseases, while the threshold II could be associated with severe (acute) diseases. In fact, the sudden collapse of the respiration rate and phosphorylation potential taking place at threshold II can serve as an energetic definition of the cell death. The death is a '0 or 1' phenomenon—there is no continuous transition between being alive and being dead. Therefore, the sharp irreversible transition occurring at threshold II seems to reflect well this property of death. Of course, this is only a rough description of the cell death in the energetic aspect—in reality certainly much more factors than just a decrease in the oxidative phosphorylation activity condition this process (e.g. free radical production).

The great difference in the behaviour of the system in Case 2 (ATP usage more sensitive to [ATP]) and Case 3 (ATP usage less sensitive to [ATP]) suggests that the differences in the K_m constant of ATP usage for ATP in different tissues may contribute to the phenomenon of tissue specificity of mitochondrial diseases. This phenomenon consists in the fact that the same degree of deficiency of some enzyme is more deleterious in some tissues (muscle, brain) than in other tissues (liver, kidney, heart). Several factors that could be responsible for this specificity have been proposed, including relative energy demand [5] and threshold values for different oxidative phosphorylation complexes [6,7]. An additional reason of the tissues specificity of mitochondrial diseases can be the difference in the sensitivity of ATP usage in different tissues to ATP (or ATP/ADP). There is no direct experimental evidence supporting this hypothesis. However, it is broadly accepted that, e.g. ATP usage in liver (especially gluconeogenesis) is very sensitive to ATP/ADP, while ATP usage in skeletal muscle is very little sensitive to ATP. This fact seems to correlate well with the fact that mitochondrial diseases are preferentially expressed in muscle in relation to liver. Generally, the merit of a high affinity of ATP usage to [ATP] is insensitivity to the energetic state of the cell, while the fault of it is the danger of non-stability and cell death.

The present work constitutes a subsequent step in the extrapolation of the properties of oxidative phosphorylation in isolated mitochondria in state 3 titrated with specific inhibitors [1,2,6,7,18] to the bioenergetic system functioning in intact tissues. In the previous articles the influence of several factors, present in intact tissues but absent in isolated mitochondria, were analysed: varying energy demand [5], varying oxygen concentration [3,4], binary ('everything or nothing') distribution of mutated enzymes among mitochondria [5] and the parallel-activation mechanism of adjusting of ATP supply to energy demand [12]. In the present article the impact on the respiration rate of another factor, namely the substrate activation by ATP that is absent in isolated mitochondria, is analysed. The theoretical simulations conducted with the aim of a computer suggest that this phenomenon may influence significantly the kinetic properties of oxidative phosphorylation, help to explain the tissue specificity of mitochondrial diseases and help to formulate a crude definition of the cell death in the energetic aspect.

It must be emphasised that a very simplified description of substrate dehydrogenation was involved in the model of oxidative phosphorylation used for the theoretical studies conducted in the present work. Therefore, the theoretical results obtained may be treated as only some semi-quantitative approximation of the complex reality. These results will have to be tested in future experimental studies.

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