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Biophysical Chemistry 96 (2002) 21–31

Biophysical Chemistry

www.elsevier.com/locate/bpc

Parallel activation in the ATP supply–demand system lessens the impact of inborn enzyme deficiencies, inhibitors, poisons or substrate shortage on oxidative phosphorylation in vivo

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Received 24 October 2001; accepted 25 January 2002

Abstract

A potential kinetic impact of parallel activation of different steps during an increased energy demand on the effect of inborn enzyme deficiencies, physiological inhibitors, external poisons and substrate shortage on oxidative phosphorylation was studied in the theoretical way. Numerical simulations were performed with the aid of the previously developed computer model of oxidative phosphorylation. It was demonstrated that the parallel activation mechanism diminishes significantly changes in fluxes and metabolite concentrations occurring at a given degree of inactivation of the system by one of the above-mentioned factors. It was also shown that parallel activation decreases greatly the threshold value of the relative activity of oxidative phosphorylation, below which the oxygen consumption flux and ATP turnover flux become significantly affected. Finally, computer simulations predicted that parallel activation leads to a considerable increase in the apparent affinity of oxidative phosphorylation to oxygen, which delays the effect of inhibitors and poisons competing with oxygen for the active centre of cytochrome oxidase. It is concluded that one of possible functions of parallel direct activation of different steps of oxidative phosphorylation is to increase the resistance of the system to a decrease in the concentration/activity of different oxidative phosphorylation complexes. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Computer model; Mitochondrial diseases; Oxidative phosphorylation inhibitors; Regulation of metabolism

1. Introduction

Oxidative phosphorylation is the main process responsible for energy production in the form of ATP in most animal tissues under most conditions. Three main mechanisms responsible for adjusting the rate of energy supply by this process to a current energy demand in intact cells have been proposed in the literature. According to the original proposition by Chance and Williams [1,2], only ATP usage (output of the system) is directly activated by some external effector, while substrate dehydrogenation and different steps of oxidative phosphorylation are activated only indirectly, via a negative feedback involving changes in different intermediate metabolites (e.g. ATP/ADP, NADH/NAD^+, Δp) (output activation mechanism). The

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PII: S0301-4622(02)00033-9

discovery of the activation in vitro of three TCA cycle dehydrogenases by calcium ions prompted several authors [3,4] to postulate that substrate dehydrogenation (input of the system) is directly activated in parallel with ATP usage, while oxidative phosphorylation is stimulated indirectly by a decrease in ATP/ADP and/or increase in $NADH/NAD^+$ (input/output activation mechanism). Finally, quantitative theoretical studies performed with the aid of the computer model of oxidative phosphorylation developed previously [5] led to the conclusion that only a direct parallel activation of ATP usage, substrate dehydrogenation and (almost) all steps of oxidative phosphorylation can account for the changes in fluxes and metabolite concentrations observed in experimental investigations during resting state → active state transition [6,7] (parallel activation mechanism). The physical factor that activates different oxidative phosphorylation complexes still remains to be identified, although calcium ions seem to activate at least some of these complexes. Nevertheless, at the present state of knowledge, the parallel activation mechanism seems to fit best to the broad set of experimental data available in the literature [8]. There is a growing body of experimental evidence that (different steps of) ATP supply is (are) directly activated when oxidative phosphorylation is stimulated by hormones or during muscle contraction ([9–12], see also discussion in [8]).

Two main profits of the postulated parallel activation mechanism have been proposed. Firstly, it ensures that the concentration of different intermediate metabolites (ATP/ADP, $NADH/NAD^+$, Δp, acetyl-CoA) is stable. Many reactions and processes in a cell use these metabolites and significant variations in their level could disturb these processes and eventually lead to cell death. This profit seems to refer to all tissues under most conditions [12–14]. Secondly, a direct activation of oxidative phosphorylation can increase the maximal capacity of this process for ATP production and thus elevate ATP turnover. This profit is less obvious, since in most tissues—such as heart and liver—the maximal respiration rate in vivo seems to match quite well the maximal oxygen consumption in isolated mitochondria [15]. On the other hand, the oxygen consumption in skeletal muscle during maximal exercise seems to be two to four times greater than in isolated skeletal muscle mitochondria [16]. In this case, therefore, the parallel activation mechanism helps to generate a possibly great power output in extreme situations.

Additionally, parallel activation of different steps can shorten transition times between different steady-states, which can help tissues and whole organisms to react to new conditions as quickly as possible.

However, oxidative phosphorylation is subject not only to physiological regulation during periods of varying energy demand, but can also be exposed to different undesired perturbations. For example, mutations in either mitochondrial or nuclear genes can lead to dysfunction or lack of several mitochondrial complexes in some fraction of mitochondria. This can lead to a decrease in the oxygen consumption and ATP turnover fluxes as well as in the phosphorylation potential, and to an increase in free radical production, which, in turn, can contribute to the etiology of mitochondrial diseases [17]. The dependence of the respiration rate (and phosphorylation potential) on the relative activity of particular oxidative phosphorylation complexes exhibits a characteristic threshold value of enzyme activity/concentration, above which the respiration rate is not affected significantly and below which it decreases rapidly [18]. It has been proposed recently [19] that random distribution of mtDNA to daughter mitochondria during mitochondria divisions leads after a few divisions to the origination of only two pure mitochondria lines: one containing only wild-type mtDNA molecules and one containing only mutated mtDNA molecules (the so-called ‘binary mitochondria heteroplasmy’). In the case of mutations completely inactivating some mtDNA-encoded oxidative phosphorylation enzyme, this leads to complete inactivation of the ‘mutated’ line (fraction) of mitochondria, which is simply equivalent to a decrease of the amount of working mitochondria [19]. The effect of such a mutation is much more pronounced than the effect of a mutation in nuclear DNA, giving a homogeneous distribution of mutated protein molecules among different mitochondria: mtDNA mutations are characterised by a

higher threshold value at the same degree of a given complex deficiency than nuclear DNA mutations, and the threshold value is identical for each complex, when plotting the flux dependency on the step activity [19]. In the case of the binary mitochondria heteroplasmy, a given degree of some complex deficiency leads to the same degree of inactivation of the entire oxidative phosphorylation system. This fact increases the harmful effect of mtDNA-dependent enzyme deficiencies on the oxidative phosphorylation system and can play an important role in the genesis of the symptoms of mitochondrial diseases.

A very characteristic property of the effect of inborn deficiencies of mitochondrial complexes is that there can be no effect on the rate of oxygen consumption and ATP synthesis, and consequently no pathological symptoms, even if only a small fraction (5–30% of the normal value) of wild-type mtDNA and enzyme molecules is present in a cell (see [17] for a review). On the other hand, at least in some cases, there seems to be only a very small excess capacity of oxidative phosphorylation in intact cells and tissues. Villani and Attardi [20] found only a small excess capacity of cytochrome oxidase in osteosarcoma cells. In a much more physiological system of a heart in situ Mootha et al. [15] observed that an exercising heart can utilise 80–90% of the maximal oxidative capacity of isolated heart mitochondria. The question arises how this fact can be reconciled with the lack of any metabolic effect (especially a disease) when the activity of oxidative phosphorylation drops below, say, 50 or 30% of its normal value. Even more dramatic situation prevails in skeletal muscle, where oxygen consumption in vivo exceeds significantly the oxidative capacity of isolated muscle mitochondria [16]. In this case, a 'disease' should develop even at normal level of wild-type mtDNA and mitochondrial enzyme molecules, unless some mechanism preventing this is present.

For the above-discussed reasons, every mechanism, which leads to an increase in the capacity of oxidative phosphorylation and to stability of intermediate metabolite concentrations (especially of phosphorylation potential), would be very profitable for the compensation of the effect of inborn enzyme deficiencies. Such a mechanism would delay the manifestation of mitochondrial diseases, decreasing the threshold value in the relative activity of oxidative phosphorylation.

The discussed mechanism would also increase the resistance of oxidative phosphorylation to some physiological inhibitors (e.g. NO, a competitive inhibitor of cytochrome oxidase [21]) and different poisons which can get to an organism from its environment (KCN, CO, heavy metals [22], alcohol [23]). Finally, this mechanism would partially compensate for the shortage of different substrates of oxidative phosphorylation, especially oxygen and respiratory substrates (glucose, fatty acids).

The present article studies theoretically the effect of the parallel-activation mechanism on the compensation of the influence of different factors (inborn enzyme deficiencies, inhibitors, poisons, substrate shortage) that cause undesired inactivation of oxidative phosphorylation, and it does so using the computer model of oxidative phosphorylation in muscle mitochondria developed previously [5,6]. These studies lead to the conclusion that parallel activation of different steps delays the decrease in the respiration (and ATP synthesis) rate and phosphorylation potential brought about by such factors, lowers the threshold value of the relative activity of oxidative phosphorylation, increases the apparent affinity of oxidative phosphorylation to oxygen and delays the effect of respiratory substrate shortage. Therefore, this mechanism seems to exert a broad variety of different effects on kinetic properties of oxidative phosphorylation.

2. Theoretical procedures

In this study, oxidative phosphorylation is modelled in the same way as in [6]. The following enzymes/processes/metabolic blocks are taken into account explicitly within the model: substrate dehydrogenation (hydrogen supply to the respiratory chain), complex I, complex III, complex IV (cytochrome *c* oxidase), proton leak, ATP synthase, ATP/ADP carrier, phosphate carrier, adenylate kinase, ATP-usage system. 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 programmes were written in the FORTRAN programming language.

In all simulations (Figs. 1–8), different levels of energy demand were fixed by a multiplication of the value of the rate constant of ATP usage (k_{UT} in [5,6]) in resting state by a factor indicated in each case. The direct activation of ATP production within the parallel-activation mechanisms (Figs. 2, 4, 6–8) consisted in increasing by the same factor (also indicated in each case) of the resting-state rate constants of substrate dehydrogenation and all oxidative phosphorylation steps but proton leak. The dependence of ATP production on the ATP/ADP ratio (Figs. 1–4) was simulated by fixing different levels of energy demand (ATP usage activity) and recording steady-state values of ATP turnover and ATP/ADP, while the dependence of ATP consumption (Figs. 1–4) on ATP/ADP was obtained in an analogous way—by manipulating the values representing the activity of the ATP-producing block. Different relative activities of oxidative phosphorylation (x axis in Figs. 5 and 6) were fixed by a gradual decrease, in subsequent simulations, of the rate constants of all oxidative phosphorylation steps and substrate dehydrogenation from 1 (normal activity) to 0. The dependence of the respiration rate on oxygen concentration (Fig. 7) was obtained by fixing different constant oxygen concentrations between 5 and 0 μM. The effect of a competitive inhibitor of cytochrome oxidase (Fig. 7) was simulated by a threefold increase in the K_m constant of cytochrome oxidase for oxygen. Because the concentration of respiratory substrates is not taken into account explicitly within the computer model used, the effect of respiratory substrate shortage (Fig. 8) was rendered by a decrease in the effective activity (rate constant) of the substrate dehydrogenation block to the extent indicated on the x axis of Fig. 8.

General theoretical results similar to, but not identical with those presented in Figs. 1–6 can be obtained with an aid of a much simpler model, involving only two processes: ATP production (kinetic expression: $v_{PROD}=k_{PROD}/(1+25\ \mu M/[ADP])$) and ATP consumption (kinetic expression: $v_{CONS}=k_{CONS}/(1+150\ \mu M/[ATP])$), with $[ATP]+[ADP]=2000\ \mu M$ (data not shown). However, such an oversimplified model is not able to produce Figs. 7 and 8.

3. Theoretical results and discussion

Figs. 1 and 2 present a comparison of the output-activation mechanism and parallel-activation mechanism of regulation of oxidative phosphorylation during an increase in energy demand in a cell. The comparison is performed in the context of changes in fluxes and metabolite concentrations during transition from resting steady-state to active steady-state. For simplicity, only two subsystems are distinguished: ATP production (substrate dehydrogenation + oxidative phosphorylation) and ATP consumption, as well as one pair of metabolites: ATP/ADP, constituting the main kinetic 'connection' between these subsystems. Simulated dependencies of ATP production and ATP consumption on the ATP/ADP ratio in resting state and active state are presented. The intersection points between these two dependencies before and after an increase in energy demand correspond to resting and active steady-states.

In the case of the output-activation mechanism (Fig. 1), only ATP consumption is activated directly by some external effector (eight times in the case of simulations presented in Fig. 1), while ATP production is stimulated only indirectly, via a decrease in the ATP/ADP ratio. In other words, the dependence of ATP usage on ATP/ADP in active state is represented by a new curve, situated eight times 'higher' than the curve for ATP consumption in resting state, while the dependence of ATP production on ATP/ADP is represented by the same curve both in resting state and in active

state. It can be seen that this kind of adjusting of the rate of ATP production to the current energy demand must involve large changes in the ATP/ADP ratio, since this is the only way to increase significantly the oxidative phosphorylation flux.

In the case of simulations concerning the parallel-activation mechanism (Fig. 2), it was assumed that ATP production was directly activated five times in parallel with the eightfold activation of ATP consumption occurring in the output-activation mechanism. Therefore, active ATP production is represented here by a new curve, situated five times 'higher' than the resting ATP production curve. The resting steady-state is of course identical for both mechanisms. In the case of parallel activation, however, the active steady-state is located in a completely different place than in the case of output activation. In the former case only a very small change in the ATP/ADP ratio takes place, while ATP turnover increases slightly more than in the latter case. It is so, because ATP production here is stimulated mainly in a direct way, while the indirect activation by a decrease in the ATP/ADP ratio plays only a secondary, 'fine-tuning' role.

Figs. 3 and 4 present a comparison of the effect of a partial inhibition of the overall ATP production (oxidative phosphorylation) activity by some factor (inborn enzyme deficiency, physiological inhibitor, poison) in the case of output activation and parallel activation. It is assumed in both cases, that the factor under consideration decreases the activity of ATP production twice. The effect of this inhibition on the behaviour of the system in active state is analysed. In the case of output activation (Fig. 3), a twofold decrease in the ATP production activity has a great impact on the active steady-state: ATP turnover decreases twice while ATP/ADP decreases over ten times in relation to normal conditions (ATP production not inhibited). A completely different situation takes place in the case of parallel activation (Fig. 4). Here, essentially no changes in the flux can be observed, while the ATP/ADP ratio drops rather insignificantly. Therefore, the parallel-activation mechanism essentially delays the effect of enzyme deficiencies, inhibitors and poisons on the bioenergetic system of a cell—a much higher degree of inhibition of oxidative phosphorylation would be required to disturb significantly the functioning of the system.

It must be emphasized that the difference between the situations presented in Figs. 3 and 4 depends to a large extent on the difference between the relative elasticity of ATP supply and ATP demand to the ATP/ADP ration. In the case of (almost) maximal energy demand at the output-activation mechanism, presented in Fig. 3, the capacity of oxidative phosphorylation for ATP production is almost saturated and its elasticity to the ATP/ADP ratio is low. In the case of a lower (say: half-maximal) energy demand, where ATP supply is much more sensitive to ATP/ADP, the difference between Figs. 3 and 4 would be essentially smaller, although it would not vanish completely. Therefore, the discussed effect of parallel activation is most pronounced at high energy demands, approaching the capacity of oxidative phosphorylation in isolated mitochondria.

Figs. 5 and 6 present a comparison of the dependence of the threshold value of oxidative phosphorylation activity on the level of energy demand in the case of output activation and parallel activation. Different energy demands were fixed by multiplying the resting activity (rate constant) of ATP usage by 1.7, 2.5, 4, 6 or 12. ATP supply was not activated in simulations for output activation, while in simulations concerning parallel activation ATP production was activated $n^{0.8}$ times, where n stands for the activation of ATP consumption in a given simulation. Maximal ATP turnover in isolated mitochondria and maximal ATP turnover in the exercising heart—equal to approximately 85% of oxidative phosphorylation capacity in isolated mitochondria [15]—are also presented in Figs. 5 and 6.

Fig. 5 demonstrates, that in the case of output activation the threshold value depends essentially (in a linear manner) on a relative energy demand: the higher the demand, the higher the threshold value. This type of behaviour was predicted previously [19] and related to the tissue specificity of mitochondrial diseases, consisting in a preferable manifestation of diseases in tissues with the highest relative energy demand, such as skeletal muscle or brain. However, it can be seen from Fig. 5 that

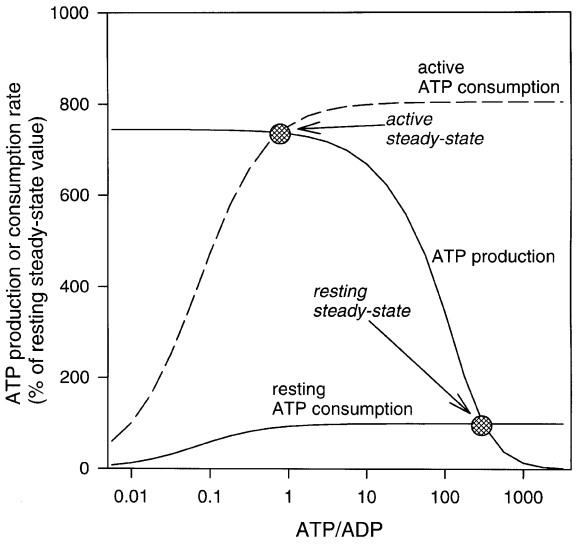

Fig. 1. Graphical presentation of the output-activation mechanism. Only ATP consumption is directly activated (eight times) during transition from resting state to active state. A large change in ATP/ADP takes place.

the maximal ATP turnover (and thus phosphorylation potential) in heart should be affected essentially, when the activity of oxidative phosphorylation decreases below approximately 80% of its normal value. This seems to remain in contradiction with the fact that a decrease of oxidative phosphorylation activity below, say, 50 or 30% of its normal value does not cause any noticeable physiological effects ([17], compare also [19]).

Fig. 6 presents a possible explanation of this apparent paradox. Here, in the case of the parallel-activation mechanism, the threshold values of the relative activity of oxidative phosphorylation are generally much smaller than in the case of output activation. Additionally, the threshold values for different energy demand levels are similar to each other, ranging from 15 to 25% of the normal activity of oxidative phosphorylation. In this case, the maximal ATP turnover in working heart would be disturbed, when the activity of oxidative phosphorylation dropped below 20% of its normal value. Therefore, the parallel activation mechanism increases greatly the effective excess capacity of oxidative phosphorylation for an increase in the ATP synthesis flux.

Fig. 7 presents a simulated effect of parallel activation on the apparent affinity of oxidative phosphorylation to oxygen, and consequently on the resistance of the system to inhibitors and poisons, such as NO and CO, which compete with oxygen for the binding site in cytochrome oxidase. It can be seen that parallel activation decreases the apparent K_m constant (or, in fact, $K_{0.5}$ constant) of oxidative phosphorylation for oxygen by approximately three times, from 1 to 0.3 μM. Therefore, in the presence of the parallel-activation mechanism, oxidative phosphorylation is less sensitive to oxygen shortage than in the presence of the output-activation mechanism. Additionally, this automatically makes oxidative phosphorylation more resistant to competitive inhibitors of cytochrome oxidase. Fig. 7 presents the results of simulations for both output activation and parallel activation, in which it was assumed that a given dose of a competitive inhibitor/poison increases three times the intrinsic K_m constant of cytochrome oxidase for oxygen. It can be seen that due to the

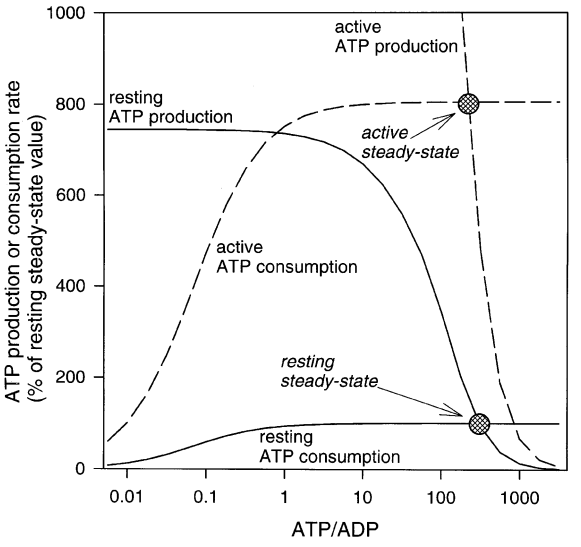

Fig. 2. Graphical presentation of the parallel-activation mechanism. Both ATP consumption and all steps of ATP production are directly activated (eight and five times, respectively) during transition from resting state to active state. A small change in ATP/ADP takes place.

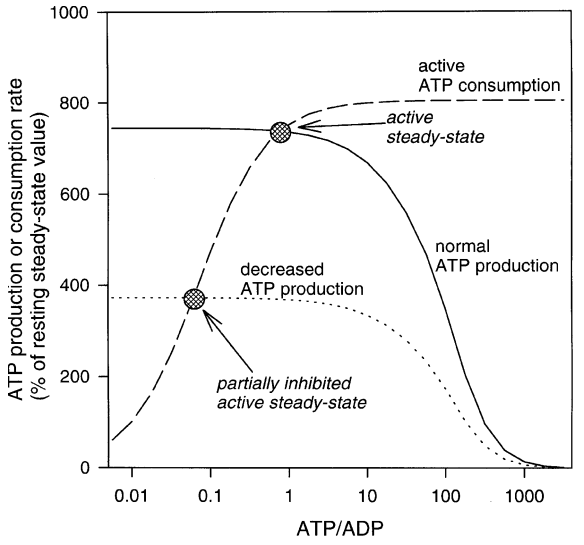

Fig. 3. Simulated effect of a twofold inactivation of (all steps of) ATP production in active state in the case of the output-activation mechanism. A large decrease in ATP production and ATP/ADP takes place.

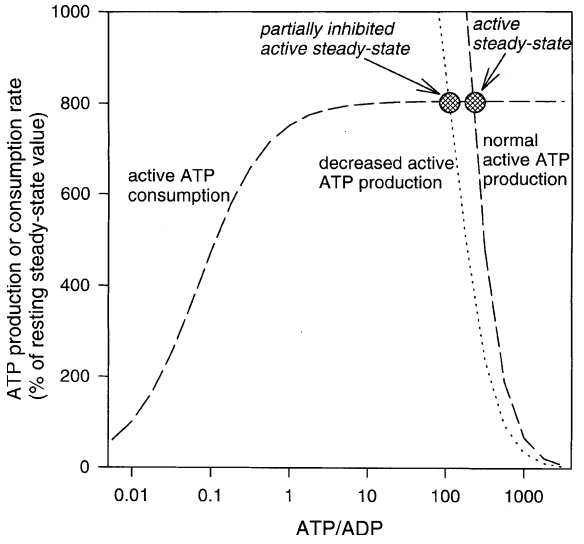

Fig. 4. Simulated effect of a two-fold inactivation of (all steps of) ATP production in active state in the case of the parallel-activation mechanism. During transition to active state, both ATP consumption and all steps of ATP production were directly activated (eight and five times, respectively), as in the simulations presented in Fig. 2. A small decrease in ATP production and ATP/ADP takes place.

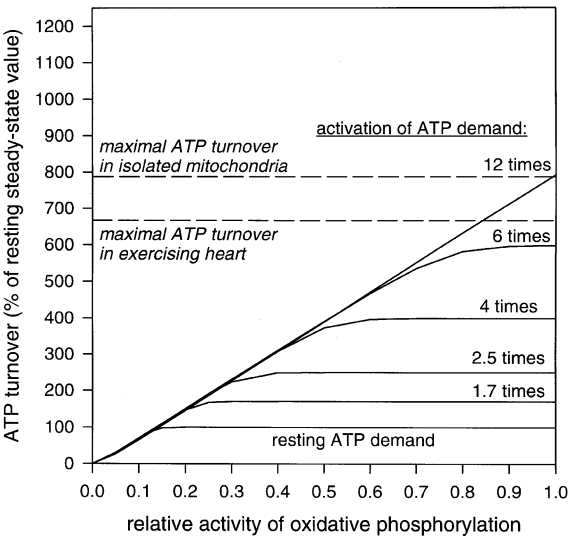

Fig. 5. Simulated dependence of ATP turnover on the relative activity of oxidative phosphorylation for different energy demands in the case of the output-activation mechanism. The threshold value increases linearly with the energy demand.

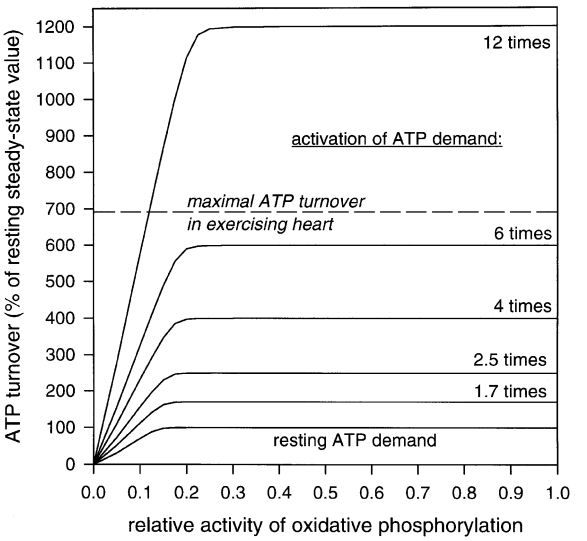

Fig. 6. Simulated dependence of ATP turnover on the relative activity of oxidative phosphorylation for different energy demands in the case of the parallel-activation mechanism. All steps of ATP production were activated $n^{0.8}$ times, where n stands for the activation of energy demand. The threshold value is essentially independent of the energy demand.

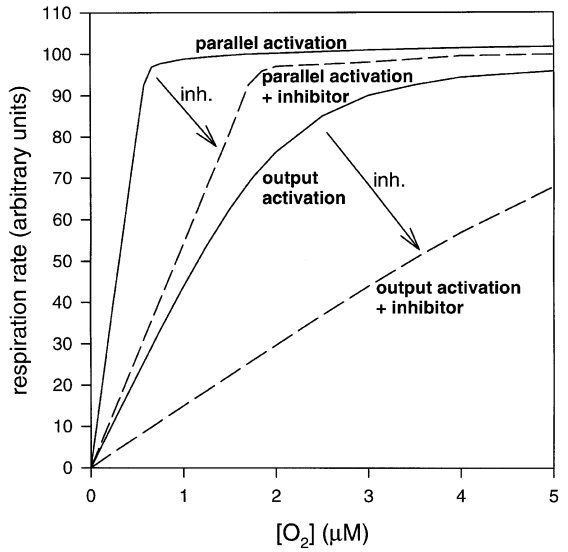

Fig. 7. Simulated dependence of the respiration rate on oxygen concentration in active state for output activation and parallel activation, in the absence (solid lines) and presence (dashed lines) of a competitive inhibitor of cytochrome oxidase. It was assumed that some dose of inhibitor decreases the intrinsic K_m constant of cytochrome oxidase for oxygen three times. Active state was achieved by either a fivefold activation of ATP consumption (output activation) or a fivefold activation of ATP consumption and $5^{0.8}$-fold activation of (all steps of) ATP production (parallel activation).

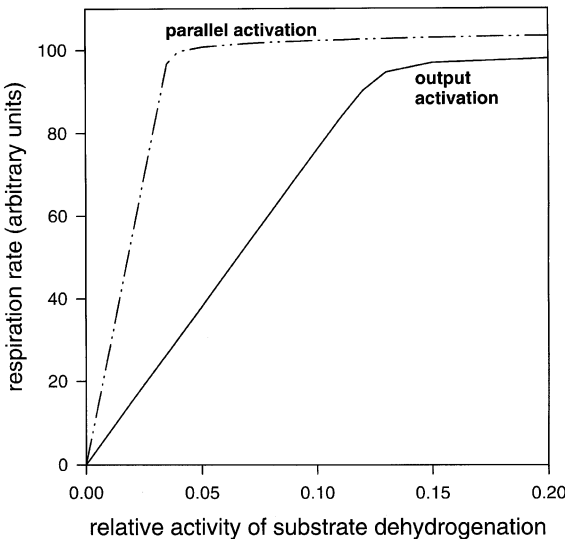

Fig. 8. Simulated dependence of the respiration rate in active state on the relative activity of substrate dehydrogenation for output activation and parallel activation. The active state was achieved by either a fivefold activation of ATP consumption (output activation) or a fivefold activation of ATP consumption and $5^{0.8}$-fold activation of (all steps of) ATP production (parallel activation).

fact that parallel activation increases the apparent affinity of oxidative phosphorylation to oxygen, this affinity is also much greater in the case of parallel activation than in the case of output activation in the presence of an inhibitor ($K_{0.5}$ equal to 0.8 and 3 μM, respectively). Again, the effect of factors decreasing the effective activity of oxidative phosphorylation is delayed, if a direct activation of different steps of oxidative phosphorylation by an external effector takes place.

Fig. 8 presents the effect of parallel activation on the response of oxygen consumption by oxidative phosphorylation to a decrease in the effective activity of the substrate dehydrogenation block caused by respiratory substrate shortage. The concentration of respiratory substrates is not taken into account explicitly in the model used for simulations. However, from the formal point of view, this effect can be rendered by a decrease in the rate constant of substrate dehydrogenation (see Section 2). It can be seen that, in the case of parallel activation, the respiration rate begins to decrease significantly at an essentially higher degree of inactivation of substrate dehydrogenation than in the case of output activation. Therefore, the parallel-activation mechanism also diminishes perturbations in the functioning of the bioenergetic system coming from respiratory substrate shortage.

Generally, all the above presented theoretical results suggest that the parallel-activation mechanism delays the effect of different factors that decrease the activity of oxidative phosphorylation, such as inborn enzyme deficiencies, physiological inhibitors, external poisons and substrate shortage. Namely, direct stimulation of different steps counteracts a decrease in fluxes and intermediate metabolite concentrations, decreases the threshold value of relative oxidative phosphorylation activity, increases the apparent affinity of the system to oxygen, delays the effect of competitive inhibitors of cytochrome oxidase and compensates respira-

tory substrate shortage. Therefore, the discussed regulatory mechanism is important not only for adjusting ATP supply to energy demand during resting state→active state transition, but also for counteracting undesired perturbations that partially inactivate oxidative phosphorylation.

The parallel activation mechanism was proposed mainly on the basis of theoretical interpretation of experimental data [6–8]. However, there are accumulating experimental observations that directly support the idea of parallel activation of (different steps of) ATP production and ATP consumption during an increased energy demand [9–12]. Nevertheless, further experimental studies are needed to decide if oxidative phosphorylation complexes are directly activated in vivo, and to what extent. Demonstrating possible profits of parallel activation can encourage people to perform appropriate experimental studies.

It is assumed in the simulations performed in the present article, 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 [19]. However, most factors (nuclear DNA mutations, inhibitors, poisons) affect selectively only some mitochondrial enzymes; additionally, inactivated complexes are in this case homogeneously distributed among mitochondria. On the other hand, if the oxidative phosphorylation system is treated as a black box (one is not interested how oxidative phosphorylation is inhibited)—as it is done in Figs. 1–6—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). Appropriate computer simulations (data not shown) confirmed this conclusion.

It is also assumed that, within the parallel activation paradigm, all oxidative phosphorylation complexes are directly activated to the same extend by some external effector during resting state→active state transition. If it appears that only some of them are in fact activated (or that some are less activated than others), then, within the black-box description, the overall effect will be equivalent to a smaller general activation of oxidative phosphorylation (ATP production). However, the general effect demonstrated in Figs. 1–6 will still remain valid. This was confirmed by computer simulations as well (data not shown).

Generally, the theoretical studies discussed in the present article are not intended to reflect any particular physiological case in a strictly quantitative way. The assumed changes in ATP turnover and ATP/ADP during an increased energy demand correspond roughly to the situation prevailing in heart and skeletal muscle in vivo [13,14]. Nevertheless, general, semi-quantitative effects and conclusions are most important. They suggest clearly an important role of parallel activation in delaying the effect of different factors that decrease the overall activity of the entire oxidative phosphorylation system.

The etiology of mitochondrial diseases was related to the threshold effect in the dependence of ATP synthesis on different mitochondrial complex activities [18], although other factors (e.g. free radicals production) can also contribute to the manifestation of oxidative phosphorylation enzyme deficiencies [17]. This proposition, although very important, was based on inhibitor titration of different complexes in isolated mitochondria, giving a homogeneous distribution of inhibited complex molecules among mitochondria. Subsequently, it was shown that the origin of a mutation (mitochondrial DNA vs. nuclear DNA), and therefore the distribution of defected enzyme molecules among mitochondria, has a significant impact on the expression of mutations [19]. The present article points to the role of the parallel-activation mechanism, absent from isolated mitochondria, in the etiology of mitochondrial diseases. Therefore, it constitutes a next step in the progress of under-

standing of the genesis of mitochondrial diseases, when one tries to transpose the results obtained on isolated mitochondria to more physiological conditions. In particular, the present paper offers an explanation why a decrease in the oxidative phosphorylation activity by more than 50% may have no visible physiological consequences, while a normal working heart can at the same time utilise 80–90% of oxidative phosphorylation capacity in isolated mitochondria.

There exists an interesting theoretical possibility that some mutations can lead not to a decrease in the activity of mitochondrial complexes itself, but to a decrease in their sensitivity to the external effector that normally activates them. This effect could contribute to the great variability of clinical symptoms caused by different mutations in genes encoding different subunits of mitochondrial complexes.

The theoretical prediction that parallel activation decreases significantly the apparent K_m ($K_{0.5}$) constant of oxidative phosphorylation for oxygen suggests that oxygen is not a significant factor controlling oxidative phosphorylation, as postulated in [24]. Additionally, it makes less probable that NO, a competitive inhibitor of cytochrome oxidase [21], affects significantly the activity of oxidative phosphorylation under physiological conditions. Generally, the present article suggests that the properties of oxidative phosphorylation in isolated mitochondria cannot be directly extrapolated to more physiological conditions.

The theoretical results obtained in the present work can also help to understand better the effect of external poisons affecting directly oxidative phosphorylation complexes, such as CO, KCN, heavy metals [22] and ethanol [23].

The parallel activation mechanism makes the system less sensitive (a lower elasticity coefficient to ATP/ADP) to perturbations in ATP production, since it diminishes the flux control coefficient of ATP supply at higher fluxes: unlike in the case of output activation in the vicinity of state 3, ATP usage has here most of the control over the system (flux control coefficient larger than 0.7) even at high ATP turnover fluxes [data not shown]. Metabolic control analysis [25,26] demonstrated clearly that the control over the flux exerted by the demand block is reversibly dependent on the ratio of the elasticity of the demand block to the elasticity of the supply block to the common intermediate metabolite concentration(s) at the intercept point (steady-state) (compare Figs. 1–4). The problem of the elasticity of the demand block has already been set up by Thomas and Fell [27]. The authors demonstrated that no realistic kinetics of the demand block allows a significant activation of this block by small changes in intermediate metabolite concentrations, because the elasticity of this block to these concentrations is too low. Consequently, the authors concluded that the 'multi-site modulation' (a parallel direct activation of different enzymes by some external effector) is a logical, if not the only, way to explain large changes in fluxes observed in intact cells and tissues, accompanied by only moderate variations in intermediate metabolite concentrations.

Perturbations in the concentration of oxygen and/or respiratory substrates can be considered as undesired (from the physiological point of view) changes which disturb the functioning of the system, and therefore low sensitivity of the system to them seems to be profitable for proper regulation of metabolism and general homeostasis of the cell. As to NO, it is not clear if its inhibitory properties play purposeful physiological role, or if they are undesired 'side effect' of the hormonal action of nitric oxide. Therefore, it is not obvious at all that sensitivity of oxidative phosphorylation to [NO] may be advantageous for the system.

To sum up, the present article demonstrates that the parallel-activation mechanism not only helps to keep intermediate metabolite concentrations as constant as possible, increases the capacity of oxidative phosphorylation and shortens transition times between different steady-states, but also delays the effect of inborn enzyme deficiencies, physiological inhibitors, external poisons and substrate shortage. Therefore, parallel activation seems to be a universal mechanism that is responsible for maintaining homeostasis in a cell.

Acknowledgments

The author is grateful to Jean-Pierre Mazat and Thierry Letellier for a stimulating discussion. This

work was supported by the KBN grant 0450/P04/2001/20.

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