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RATE-CONTROLLING STEPS OF OXIDATIVE PHOSPHORYLATION IN RAT LIVER MITOCHONDRIA

A SYNOPTIC APPROACH OF MODEL AND EXPERIMENT

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The contribution of different steps to the control of oxidative phosphorylation in isolated rat liver mitochondria was investigated by a combination of experiments and computer simulations. The parameters of the mathematical model of phosphorylating mitochondria were derived from experimental data. The model correctly describes the competition between ATP utilization inside and outside mitochondria for the ATP generated in mitochondria. On the basis of the good agreement between experiments and simulations, the contribution of different steps to the control of respiration was estimated by computing their control strengths, i.e., the influence of their activities on the rate of respiration. The rate-controlling influences vary depending on the load of oxidative phosphorylation. The predominant steps are: in the fully active state (State 3)—the hydrogen supply to the respiratory chain; in the resting state (State 4)—the proton leak of the mitochondrial inner membrane; in states of non-maximum ATP export—the adenine nucleotide translocator. Titrations of respiration with phenylsuccinate, antimycin, oligomycin and carboxyatractyloside completely support these conclusions.

Introduction

Starting with the well known phenomenon that ADP accelerates mitochondrial respiration [1] and proceeding to the detection of respiratory control by the extramitochondrial ATP/ADP ratio [2–4], many experiments were done to understand the regulation of oxidative phosphorylation. Most interpretations of the measured data were based on a more or less static aspect, focussing interest on a single reaction step, which should be responsible for the control over the whole range of activity. Erecińska and Wilson [5], for instance, consider the cytochrome oxidase as the only control point, the activity of which is linked to the intramitochondrial NADH/NAD ratio and to the extramitochondrial mass-action quotient ([ATP]/([ADP]·[P_i]) by near-equilibrium processes.

From the physiological point of view such a rigid organization of the regulation seems not to be reasonable, since it would not allow the cell to react to the variety of situations which occur in vivo. Therefore, we applied the concept of control strength [6] (or sensitivity, cf. Ref. 7) to the problem of respiratory control in order to evaluate the importance of different reaction steps under conditions of varying energy demands. For this purpose, we combined the experimental investigation of isolated mitochondria with the simulation of such experiments by a mathematical model [8]. The model permits us to calculate control strengths as a measure of the influence caused by the activ-

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ity of single reaction steps on the respiration rate. Experimental estimates of the control strengths were obtained by inhibitor titrations. The model predictions and the experimental results were found to be in complete agreement. The close congruence justifies the prediction from model simulations of the relationship of the adenine nucleotides on both sides of the mitochondrial membrane under conditions of different energy drain, which could not be experimentally investigated for technical reasons. It is demonstrated that the importance of the single steps for the control of oxidative phosphorylation changes over the whole range of activity with maximum influence of the hydrogen supply in the fully active state (State 3), of the adenine nucleotide translocator in the control range, and of the proton leak in the resting state (State 4).

Methods

Experimental procedures

Isolation of mitochondria from rat liver and the determinations of mitochondrial protein, adenine nucleotides and respiration rates were performed as described earlier [2]. For experiments on citrulline synthesis, mitochondria were taken from rats fed for 4–6 days with a high-protein diet [9] and

TABLE I

EQUATIONS OF THE MATHEMATICAL MODEL

v_h, v_r, v_o, v_a, v_t, v_l, v_i, v_e and v_{ex} —rates of: hydrogen supply, electron flux through the respiratory chain, terminal oxidation, ATP formation, adenine nucleotide transport, proton leak, internal and external ATP utilization, and net production of external ATP, respectively. a_{ox} and d_{red}, mole fractions of the oxidized primary hydrogen acceptor and of the reduced final electron donor in the respiratory chain, respectively. $\Delta\mu$, proton electrochemical gradient; $\Delta\psi$, membrane potential; $Z = 60$ mV; ϕ_P, ratio of the mole fractions of monovalent phosphate in the intra- and extramitochondrial compartments; subscripts I and E, intra- and extramitochondrial compartments, respectively. The parameters (V_h, n_r, $\Delta\mu_r$, etc.) are explained in Table II.

Eqn. No.	Equations
Rate equations	
(1)	$v_h = V_h \cdot a_{ox}$
(2)	$v_r = V_r \left(1 - \frac{a_{ox}}{1 - a_{ox}} \cdot \left(\frac{d_{red}}{1 - d_{red}}\right)^2 10^{-n_r(\Delta\mu \quad \Delta\mu_r)/Z}\right)$
(3)	$v_o = V_o \cdot d_{red}$
(4)	$v_a = V_a \left(1 - \frac{[ATP]_I}{[ADP]_I \cdot [P_i]_I} \cdot \phi_P \, 10^{n_a(\Delta\mu \quad \Delta\mu_a)/Z}\right)$; $\phi_P = f_P + (1 - f_P) \cdot 10^{\Delta pH}$; $\Delta pH = -f\mu \cdot \Delta\mu / Z$; $[P_i]_I = [P_i]_E \cdot \phi_P \cdot 10^{\Delta pH}$
(5)	$v_t = V_t \frac{1 - \frac{[ATP]_E}{[ADP]_E} \cdot \frac{[ADP]_I}{[ATP]_I} \cdot 10^{\Delta\psi/Z}}{\left(1 + \frac{[ATP]_E}{[ADP]_E} \cdot 10^{f_t \cdot \Delta\psi/Z}\right)\left(1 + \frac{[ADP]_I}{[ATP]_I}\right)}$; $\Delta\psi = \Delta\mu + Z \cdot \Delta pH$
(6)	$v_l = k_l \cdot 10^{-\Delta\mu/Z}$
(7)	$v_i = k_i([ATP]_I / [ADP]_I)$
(8)	$v_e = V_e \frac{[ATP]_E}{[ATP]_E + [ADP]_E}$
Steady-state conditions	
(9, 10)	$v_h = v_r = v_o$
(11)	$n_r v_r = n_a \cdot v_a + v_t + v_l$
(12)	$v_a = v_i + v_t$
(13)	$v_t = v_e + v_{ex}$

incubated in a saline medium consisting of 50 mM KCl, 20 mM $KHCO_3$, 5 mM KH_2PO_4, 15 mM glucose, 2 mM $MgCl_2$, 0.5 mM EDTA, 25 mM Tris-HCl, pH 7.2, gassed with 95% O_2 plus 5% CO_2. The inhibitor titrations were carried out in a sucrose medium containing 110 mM sucrose, 60 mM KCl, 15 mM glucose, 10 mM KH_2PO_4, 5 mM $MgCl_2$, 0.5 mM EDTA, 60 mM Tris-HCl, pH 7.4. All experiments were performed at 25°C.

Mathematical model

The whole set of equations used for the simulation of stationary states of mitochondrial energy transformation is presented in Table I. Most of the equations are the same as those published in an earlier paper [8], where also a detailed description of the model was given. The rate equation for adenine nucleotide exchange was changed as explained in Ref. 10. Two additional equations describe the rate of internal ATP utilization by citrulline synthesis (Eqn. 7) and the splitting of external ATP by ATPase activities present in mitochondrial preparations (Eqn. 8). The proportionality between the rate of citrulline synthesis and the internal ATP/ADP ratio expressed by Eqn. 7 was demonstrated in experiments by Williamson et al. [11].

The model parameters together with their refer-

TABLE II

PARAMETERS OF THE MODEL

The values were selected for the simulation of energy transformation in rat liver mitochondria oxidizing glutamate (data in brackets refer to succinate in the presence of rotenone) at 25°C. Data without references were estimated from experiments in our laboratory.

Parameter		Value	Basis
Stoicheiometry			
n_r	(total H^+ of all coupling sites/$2e^-$)	9 (6)	thermodynamic estimations [12,13]
n_a	(H^+/ATP)	2	necessity of an additional transport proton for exchange of ATP, ADP and P_i (cf. Ref. 14)
Thermodynamics: proton driving forces under standard conditions (mV)			
$\Delta\mu_r$	(respiratory chain)	−150 (−125)	$E_0' = -320$ mV for NADH [15] (5 mV for ubiquinone [16]) and $E_0' = 380$ mV for cytochrome a_3 [17]
$\Delta\mu_a$	(ATPase)	−150	Phosphorylation potential $\Delta G^0 = 29$ kJ/mol [18]
Kinetics: maximum velocities and rate constants (nmol/min per mg)			
V_h	(hydrogen supply)	90 (200)	maximum rates of substrate oxidation
V_r	(reversible electron flux)	2000	lower limit for rapid equilibrium
V_o	(oxidation of cytochrome a_3)	$1.5 \cdot 10^6$	ATP_i/ADP_i in resting state (cf. Table III)
V_a	(ATP synthesis)	2000	upper limit of 5% control strength in the active state (cf. Fig. 3)
V_t	(ATP-ADP translocation)	1000	stimulation of respiration by ADP_E up to 80% of uncoupled respiration (substrate: succinate)
V_e	(external ATP splitting)	20	stimulation of respiration in the presence of ATP_E [2]
k_l	(proton leak) [a]	0.025	respiration in the absence of external adenine nucleotides (cf. Table III)
k_i	(internal ATP utilization)	60	stimulation of respiration by synthesis of citrulline (cf. Table III)
Kinetics: suppression of ATP uptake			
f_ψ	(effective fraction of $\Delta\psi$)	0.3	10-fold faster uptake of ADP_E if $[ATP_E]=[ADP]_E$ [19]
Extramitochondrial conditions:			
$[P_i]_E$		5 mM	cf. conditions given in Methods
f_P	(mole fraction of $H_2PO_4^-$)	0.2	pH = 7.5, $pK' = 6.8$ [20]
f_μ	(contribution of ΔpH to the proton-motive force)	0.13	$[P_i]_i \approx 40$ mM in resting state [21]

[a] In Ref. 8 this parameter was denoted by V_l which was incorrect, because k_l does not have the character of a maximum velocity.

ence data [12–21] are collected in Table II. Most of the values were estimated as described previously [8,10], the others (V_o, V_a, V_t, k_i, f_μ) were obtained by trial and error from comparison of computed and reference data. Stationary flux rates, metabolite patterns and potentials were computed from chosen values of the respiration rate v_{resp} (cf. Ref. 8). For the simulation of experiments with variable proton leak or variable capacity of citrulline synthesis (i.e., k_i), the quantities $[ATP]_E/[ADP]_E$, $[ATP]_I/[ADP]_I$ and v_a were calculated by an iterative procedure for solving the equation $x = \phi(x)$ (with $x = [ATP]_E/[ADP]_E/$).

The control strengths [6] $\partial \ln v_{resp}/\partial \ln c_j$ (where c_j terms are the maximum velocities or rate constants in Table I) were approximated by difference quotients. The correctness of the calculation was checked by the sum of the control strengths, which was in all cases not significantly different from unity (summation property, cf. Ref. 7).

Results

Reflection of control phenomena by the model

The mitochondrial energy state and the flux rates of oxidative phosphorylation depend on energy-utilizing reactions as well as on the substrate, which is the fuel of mitochondrial energy transformation. This is illustrated by the changes in the adenine nucleotide patterns inside mitochondria and in the corresponding respiration rates given in Table III. When succinate (in the presence of rotenone) was the substrate, the resting rate of respiration could be doubled under conditions of citrulline synthesis. This stimulation was connected with a decrease in the internal ATP/ADP ratio. When succinate was substituted by glutamate, two differences were osberved. Firstly, the rates of respiration and the ATP/ADP ratios were always lower. Secondly, the synthesis of citrulline led to much greater changes in both the rate of respiration and the ATP/ADP ratio.

Model simulations of these experiments allow the testing whether such behaviour can be explained by the properties of the existing model [8,10]. The computed data in Table IV are in sufficient agreement with the experimental ones in Table III, particularly the results obtained for glutamate. The deviations of the respiratory rates found for succinate may be accounted for by the presence of mitochondrial fragments still containing the membranous system of succinate oxidase

TABLE III

INTRAMITOCHONDRIAL ADENINE NUCLEOTIDES AND RESPIRATION RATES UNDER RESTING AND CITRULLINE-SYNTHESIZING CONDITIONS

Liver mitochondria isolated from rats fed with a high-protein diet were incubated in a saline medium (cf. Methods) with the additions as specified. The adenine nucleotides were measured after quenching by $HClO_4$ in the total extracts. Values are given as mean ± S.D. from n measurements.

Incubation conditions	Intramitochondrial adenine nucleotides (nmol/mg protein) ($n = 6$)				Respiratory rate ($n = 3$) (nmol O_2/ min per mg protein)
	ATP	ADP	AMP	ATP/ADP	
8 mM succinate 1 μM rotenone	9.00 ± 0.54	2.00 ± 0.13	2.36 ± 0.27	4.50 ± 0.56	19.4 ± 2.7
8 mM succinate 1 μM rotenone 10 mM NH_4Cl 10 mM ornithine	8.16 ± 0.52	2.49 ± 0.14	2.69 ± 0.27	3.28 ± 0.39	39.9 ± 4.0
8 mM glutamate	8.34 ± 0.73	2.24 ± 0.16	2.18 ± 0.24	3.73 ± 0.59	7.1 ± 0.2
8 mM glutamate 10 mM NH_4Cl 10 mM ornithine	6.52 ± 0.68	3.64 ± 0.17	2.47 ± 0.14	1.97 ± 0.27	18.6 ± 0.53

TABLE IV

COMPUTED VALUES OF INTERNAL ATP/ADP RATIOS AND RESPIRATION RATES UNDER RESTING AND CITRULLINE-SYNTHESIZING CONDITIONS

The data were computed from the parameter values in Table II. Since no external ATP was present in the experiments of Table III, $V_e = 0$ was used.

Condition	ATP/ADP	Respiratory rate (nmol O_2/min per mg)
Substrate: succinate		
resting ($k_i = 0$)	6.3	13
citrulline synthesis ($k_i = 60$ nmol/min per mg)	2.2	31
Substrate: glutamate		
resting ($k_i = 0$)	3.8	7.0
citrulline synthesis ($k_i = 60$ nmol/min per mg)	1.8	17

but not the soluble glutamate dehydrogenase. For simulation of the succinate experiments only three parameters were changed: the proton stoicheiometry of the respiratory chain, the redox potential of the hydrogen acceptor, and the maximum rate of hydrogen supply. The higher internal ATP/ADP ratio found under this condition indicates a more energized state of mitochondria which is caused by the increased capacity of hydrogen supply. The accelerated respiratory rate in presence of succinate is due to two reasons. Due to the lower number of coupling sites, more oxygen must be reduced in order to yield the same amount of energy. In addition, the higher energy state produces higher rates of energy utilization by the back-flow of protons and by citrulline synthesis.

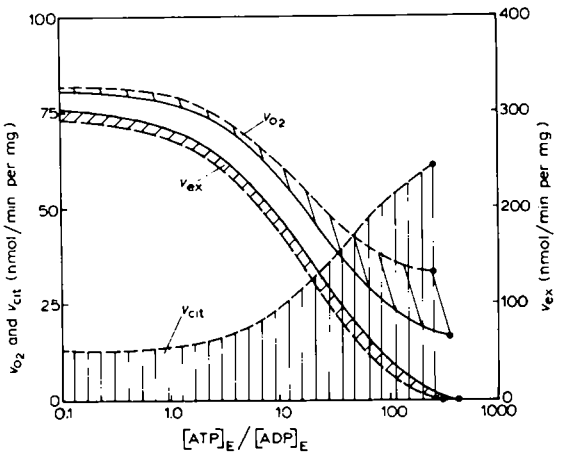

Fig. 1. Simulated competition between citrulline synthesis and net phosphorylation of extramitochondrial ADP and its effect on mitochondrial respiration. The stationary rates of respiration (v_{O_2}), of citrulline synthesis ($v_{cit} = 0.5\ v_i$) and of net phosphorylation of external ADP (v_{ex}) were computed for two conditions and plotted versus the extramitochondrial ATP/ADP ratio: (— — —) in the presence of citrulline synthesis ($k_i = 60$ nmol/min per mg); (———) in the absence of citrulline synthesis ($k_i = 0$). The other parameter values were as in Table II. The shaded areas indicates the shifts produced by citrulline synthesis.

The good agreement between experimental results and mathematical simulation justifies the model approach to a more complicated experimental situation, where extra- and intramitochondrial processes compete for the ATP generated by mitochondria. The simulated curves in Fig. 1 present the effect of the extramitochondrial ATP/ADP ratio on the rates of respiration and of phosphorylation of external ADP in the absence and presence of internal ATP turnover by citrulline synthesis. Mitochondria respond to a decreased ATP/ADP ratio by an acceleration of ATP export which activates respiration and suppresses citrulline synthesis. The most important effect demonstrated in Fig. 1 is the shift in the respiratory curve caused by citrulline synthesis. We had observed the same effect in experiments with liver mitochondria from rats fed with a high-protein diet [9]. It indicates that no fixed relationship exists between the respiratory rate and the extramitochondrial adenine nucleotide pattern. The relationship is modified by the adenine nucleotide translocator as a function of the rate of ATP-ADP exchange. The latter is lower, if ATP is

in part utilized within the mitochondria for citrulline synthesis.

Inhibitor titrations and control strengths

The importance of the control brought about by the translocator in relation to that of other control steps was evaluated by titrations of the respiratory rate with different specific inhibitors. This was done under two sets of conditions: maximum flux, i.e., in the active state; and at about half-maximum rate of respiration, adjusted by an appropriate amount of hexokinase in the glucose-containing incubation mixture.

The inhibition patterns (Fig. 2) are of three types. Phenylsuccinate (Fig. 2A), inhibiting the uptake of succinate by mitochondria, showed the strongest effect in the active state. This indicates that in this state the respiratory rate depends much more on the supply of substrate than at slower rates of respiration.

A second type of inhibition was found for antimycin A and oligomycin (Fig. 2B and C). The inhibition occurred after some threshold concentration without detectable differences between the two sets of conditions. (The absolute rates obtained with an excess of oligomycin were identical, the differences in Fig. 2C are caused by the different initial rates in absence of oligomycin.) Both the threshold behaviour and the missing influence of the activity state of mitochondria are explained in the simplest way by assuming that the reactions inhibited by antimycin and oligomycin, i.e., the electron flux through the respiratory chain and the synthesis of ATP, respectively, have a capacity exceeding that of the overall reaction of oxidative phosphorylation. Threshold effects could

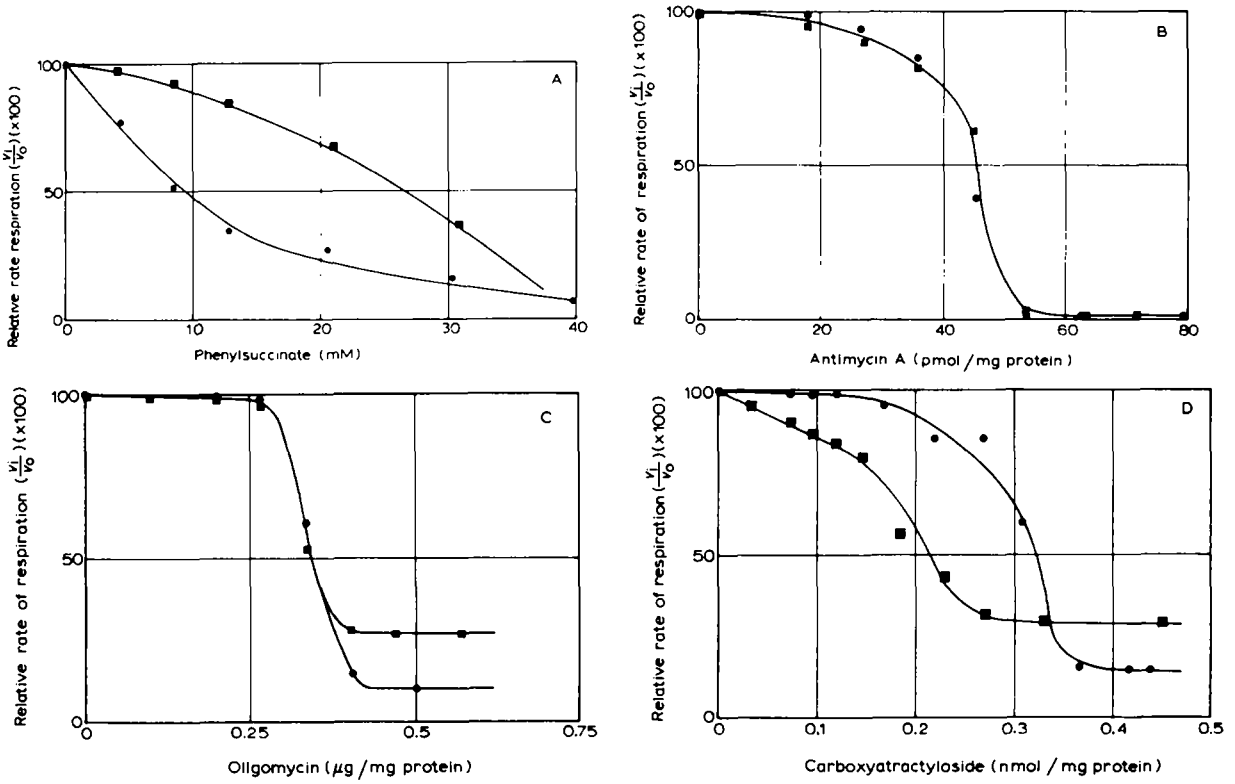

Fig. 2. Titration of the respiration of phosphorylating mitochondria with phenylsuccinate (A), antimycin (B), oligomycin (C) or carboxyatractyloside (D). Rat liver mitochondria were incubated in a sucrose medium (cf. Methods) with 10 mM succinate, 1 μM rotenone and 5 mM ATP. By the addition of hexokinase, mitochondria were brought to the fully active state (●———●) (13 nkat hexokinase/mg) or stimulated to about 50% of the maximum activity (■———■) (0.4–0.8 nkat hexokinase/mg). The rate of respiration after addition of the inihibitor (v_i) is given in per cent of the intial rate (v_0).

also be caused by cooperative binding characteristics as discussed by Slater et al. [22]. However, no cooperativity could be established either for the effect of antimycin on the oxidation of reduced ubiquinone [23] or for the inhibition by oligomycin of P_i-ATP exchange reaction or the mitochondrial ATPase [24].

Carboxyatractyloside gave a third type of inhibition pattern (Fig. 2D). Here, the effect was stronger at half-maximum rate of oxidative phosphorylation than in the active state. Therefore, the respiratory rate must be more strongly influenced by the activity of the translocator, if the net flux through the translocator is lower than that in the fully active state.

The mathematical model allows the evaluation of the importance of different control steps over the whole range of activity by computation of control strengths. Since the control strength expresses the effect on the overall rate of changes in the activity of a particular reaction step, it is proportional to the initial slopes of the inhibition curves in Fig. 2. In Fig. 3, the control strengths computed for some reactions steps were plotted versus the external ATP/ADP ratio. The corresponding flux rates are identical with those shown in Fig. 1 for the absence of citrulline formation. The most important result is that the control strengths are not fixed quantities; they depend strongly on the activity state of mitochondria. Most of them increase with the flux rates, the increase being drastic for the hydrogen input and small for the electron flux and the ATPase. The latter must be considered in relation to the general small control strength of these steps. The control strength of the leak falls with increasing rate of oxidative phosphorylation according to its share in the total energy turnover. The adenine nucleotide translocator has the greatest influence in the range of submaximum rates of respiration. Detailed investigation of the control properties of the translocator demonstrated that this behaviour is produced by the inhibitory effects of external ATP on the net exchange of ADP and ATP [10]. As the external ATP turnover increases, the ATP/ADP ratio declines and, therefore, the inhibition by ATP. It causes a decrease in the control strength in the upper range of activity.

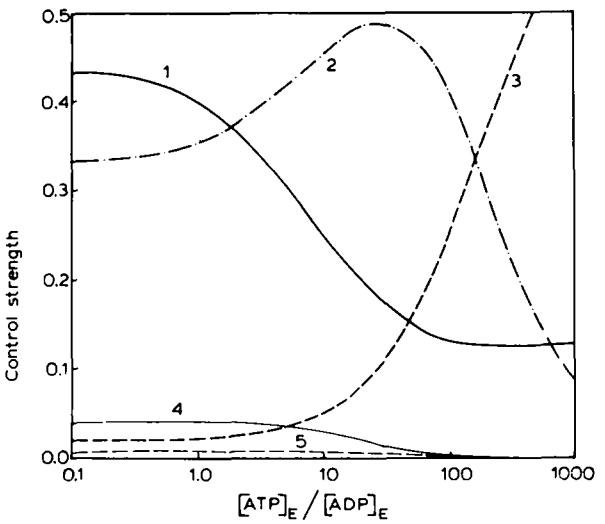

Fig. 3. Computed control strengths of different single reactions with respect to mitochondrial respiration. The control strengths were calculated for different activity states of oxidative phosphorylation and were plotted versus the extramitochondrial ATP/ADP ratio. The conditions correspond to those in Fig. 1 in the absence of citrulline synthesis. Traces: (1) hydrogen supply, (2) adenine nucleotide translocator, (3) proton leak, (4) ATPase, (5) electron flux.

The computed control strengths are in complete accordance with the inhibition patterns shown in Fig. 2 for the two different states of activity. This concerns the opposite effects in the inhibition characteristics of the substrate supply and of the adenine nucleotide translocation as well as the excess capacities deduced from the threshold behaviour of the inhibition by antimycin and oligomycin.

The model includes two further reactions for which the control strengths are not shown in Fig. 3. The value for cytochrome oxidase amounted to about 0.2 with only slight changes in the whole range of activity. The other reaction is the splitting of external ATP by enzyme activities present in mitochondrial preparations. It produces an effect on the mitochondrial respiration by decreasing the external ATP/ADP ratio and has not direct influence on the way in which mitochondria respond to this ratio. Therefore, this control strength is always zero.

Prediction of flux-dependent relationships between the ATP/ADP ratios on both sides of the mitochondrial membrane

The influence of the flux rates on the role of the

adenine nucloetide translocator in the control of oxidative phosphorylation should result in changing relationships between the external and internal adenine nucleotide patterns. This could be only partly investigated by experiments, because it was impossible to stimulate mitochondrial respiration by citrulline synthesis to more than about 60% of its maximum [25].

Since in model simulations such a restriction does not exist, it was used for further investigation of this point. It is demonstrated in Fig. 4A that the stimulation of respiration by internal ATP utilization or by direct utilization of the proton gradient (e.g., for ion translocation) leads to a smaller decrease in the external ATP/ADP ratio than that required for the same extent of stimulation by external ATP utilization. The difference between the curves obtained for utilization of internal ATP or of the proton gradient is so small that it may be difficult to detect experimentally. The response of mitochondrial respiration to the internal ATP/ADP ratio is predicted to be relatively uniform in any case (Fig. 4B). It is clear from Fig. 4A and B that the relationship between the internal and the external ratio must depend on the localization of the energy drain (Fig. 4C). If the energy supplied by respiration is utilized within mitochondria, the adenine nucleotide translocator is in thermodynamic equilibrium. Then the drop of the internal energy state is paralleled by the decrease in the external ATP/ADP ratio. The stimulation of the respiration by external ATP utilization requires a net flux through the translocator. It prevents a complete equilibration so that the internal ATP/ADP ratio cannot be lowered to the value that is in equilibrium with the external one.

Discussion

The general agreement between the results obtained from experiments and from the model leads to the conclusion that the essential properties of the energy-transforming reactions in isolated mitochondria are correctly comprised in the model. Consequently, the approximations used in the model are permissible, and the application of the model as done in this paper seems to be justified.

The results reported here lead to a coherent picture of the control steps in oxidative phosphorylation. In addition to the cytochrome oxidase, as the only controlling step considered by Erecińska and Wilson [5], there are two further kinetically important processes: the supply of substrate, and adenine nucleotide translocation. As revealed by inhibitor titrations and by the computed control strengths, the contribution of these factors in the control changes with the state of mitochondrial activity. From this point of view,

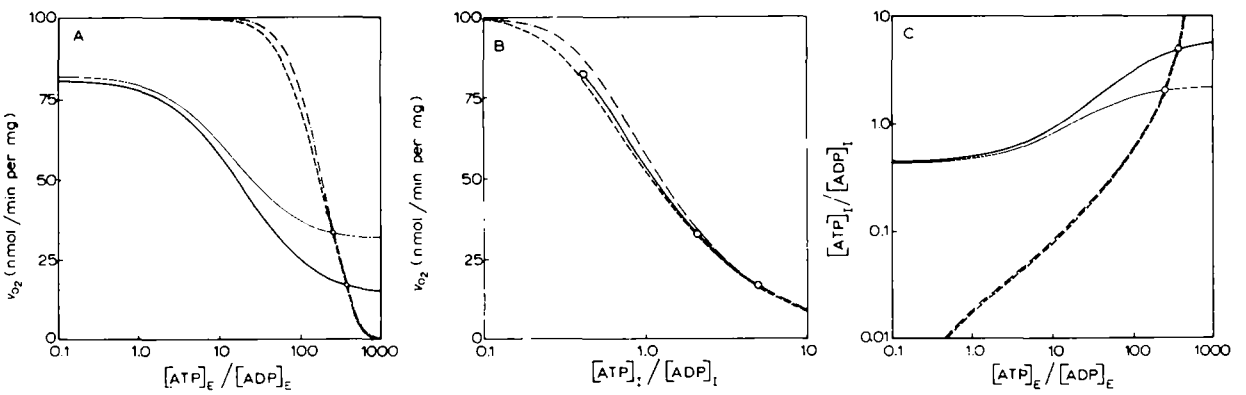

Fig. 4. Comparison of ATP/ADP ratios computed for different typs of energy drain. The relationships between respiration and external ATP/ADP (A) or internal ATP/ADP (B) and between ATP/ADP in both compartments (C) were calculated for varying net phosphorylation of external ADP (_____, without citrulline synthesis, $k_t = 0$; ———, in the presence of internal ADP turnover, $k_t = 60$ nmol/min per mg), for varying internal ATP turnover (— — —) and for varying proton leak (— · —). The points of intersection (o) denote the resting state in the absence and presence of citrulline formation, in (B) also the active state (O——) is marked.

some controversial findings may have arisen through the choice of the particular experimental conditions, promoting one of the control steps and suppressing the other. The activity of hydrogen input by substrate supply not only limits the maximum rate of respiration, but also has a finite influence in the range of lower respiratory activities. It results in substrate-dependent energy states and, consequently, different respiratory rates even under resting conditions, as demonstrated in Table III.

The high control strength of the adenine nucleotide translocator in the range of ATP/ADP-controlled respiration demonstrates that the translocator operates at some distance from equilibrium. This distance causes a loss of free energy so that the resulting external ATP/ADP ratio is smaller than the value possible under equilibrium conditions. It explains the shifts in the relationships between the external and internal ATP/ADP ratios predicted by the model (Fig. 4C). The predicted influence of the different kinds of energy drain, i.e., direct utilization of external or internal ATP or of the proton gradient, are completely supported by experiments carried out for some distinct conditions [25].

An additional conclusion is suggested by the curves in Fig. 4C. It seems impossible to decrease the internal ATP/ADP ratio below some limit by utilization of external ATP. Thus, the translocator may have a protective function, preventing the complete de-energization of mitochondria by cytosolic processes. Such a mechanism could be of importance under conditions of excessive energy demand.

The steps of energy transformation in the respiratory chain and of the ATPase seem to have a sufficient capacity for operation near equilibrium. This causes a minimum loss of free energy so that a high efficiency is made available for these energy-transforming reactions. The close equilibrium operation of the ATPase is in line with the consistently parallel changes in the proton gradient and internal ATP/ADP ratio observed under conditions of different energy drains [25]. The shifts in the relationship between respiration and the internal ATP/ADP predicted for different energy drains (Fig. 4B) are so small that it is obviously impossible to resolve them by experiments.

On the basis of these results, it seems hardly helpful to describe the control of oxidative phosphorylation by a generalized driving force derived from the overall reaction [26–28]. The total driving force results from the component forces that are necessary for the kinetic control steps. Since their contribution to the control depends on the particular conditions, these contributions to the total driving force must be changeable.

In summary, the kinetic properties of the supply with reducing equivalents, of cytochrome oxidase, and of the adenine nucleotide translocator are considered to determine, in their non-constant combination, the response of mitochondria to energy requirement. With this concept, it is possible to interpret quantitatively experimental results obtained under many different conditions in a coherent way, as demonstrated by the use of the mathematical model.

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