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CONTROL OF MITOCHONDRIAL RESPIRATION

THE CONTRIBUTION OF THE ADENINE NUCLEOTIDE TRANSLOCATOR DEPENDS ON THE ATP- AND ADP-CONSUMING ENZYMES

FRANK NORBERT GELLERICH, RALF BOHNENSACK and WOLFGANG KUNZ

Institut für Physiologische Chemie, Medizinische Akademie Magdeburg, Leipziger Str. 44, DDR 3090 Magdeburg (G.D.R.)

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The consequence of the complexity of the metabolic network on the amount of control strength of adenine nucleotide translocator was investigated with isolated rat liver mitochondria. Two experimental systems were compared: (i) mitochondria in the presence of yeast hexokinase (hexokinase system) and (ii) the same system plus additional pyruvate kinase (pyruvate kinase system). In both systems the control strength was analysed for the adenine nucleotide translocator by inhibitor titration studies with carboxyatractyloside and for the hexokinase or pyruvate kinase by changing their relative activities. Experimental results were compared with computer simulation of these systems and that of a third one, where the extramitochondrial ATP/ADP ratio was held constant by perifusion (perifusion system). The results demonstrate quite different flux-dependent control strength of the translocator in the three systems. In the hexokinase system the control strength of the translocator on mitochondrial respiration was zero up to respiration rates of about 60 nmol O_2/mg protein per min. For higher rates, the control strength increased until the maximum value (0.45) was reached in the fully active state. Here, the same value was also found in the pyruvate kinase system. In all other states of respiration the translocator exerts a higher control strength in the pyruvate kinase system than in the hexokinase system. This different behaviour was attributed to the various changes in the adenine nucleotide pattern caused by partial inhibition of the translocator in the hexokinase and pyruvate kinase system. The data clearly show that the sharing of control strength depends not only on the respiration rate but also on the complexity of the metabolic system.

Introduction

In aerobic cells both the mitochondrial H^+-ATPase and enzymes of the glycolytic pathway phosphorylate ADP. Most of the ATP-utilizing reactions are localized extramitochondrially. Both compartments found for cellular adenine nucleotides [1,2] are connected by the adenine nucleotide translocator, mediating the competition of different reactions for adenine nucleotides. The finding that in phosphorylating mitochondria the translocator operates far from equilibrium [3–6] suggests its contribution to the control of adenine nucleotide fluxes. This is in line with the dependence of mitochondrial respiration on the extramitochondrial ATP/ADP ratio [7] which was attributed to the kinetic properties of the translocator [8,9]. In contrast, Erecińska and Wilson [10] concluded that

Abbreviation: CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

the translocator does not participate in control of mitochondrial respiration. Also, at the level of whole cells, e.g., hepatocytes [11,12] or yeast cells [13], experiments on the participation of the adenine nucleotide translocator in the control of respiration lead to different interpretations. Recently, we pointed out the dependence of the control function of the adenine nucleotide translocator on the complexity of the experimental model system [3]. It was predicted that the control of the respiration rate by the translocator should be influenced by a second competing reaction of ATP formation. The concept of control strength developed by Kacser and Burns [14] and Heinrich and Rapoport [15] allows quantification of the amount of control which a particular enzyme exerts on the flux through a pathway. Using this concept it was possible to quantitate the contribution of the translocator to the respiration control [16,17]. This report is specially focused on the consequences of system complexity on sharing the control strength between ATP-generating and -utilizing processes. For this purpose the hexokinase system (Fig. 1B), consisting of mitochondrial oxidative phosphorylation and yeast hexokinase, was extended by a competing extramitochondrial ATP-regenerating system (Fig. 1C). Muscle pyruvate kinase was shown to be a suitable candidate for adjusting different stationary states of mitochondrial respiration in the presence of hexokinase [18,19]. We present an experimental investigation of the hexokinase and pyruvate kinase systems in comparison with computer simulations of these and the perifusion system (Fig. 1A). The results demonstrate quite different flux-dependent control strengths of the translocator in the three cases. The pyruvate kinase system seems to be a suitable experimental model for simulating properties of energy metabolism in intact cells. The consequences of our findings for the interpretation of results obtained in intact cells [11–13] will be discussed.

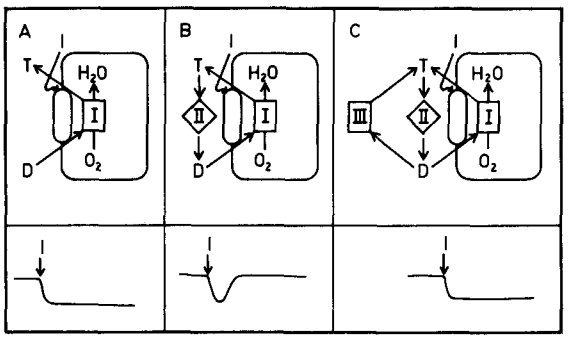

Fig. 1. Effect of partial inhibition of the adenine nucleotide translocator on the respiration rate in systems of different complexity. (A) Mitochondrial ATP regeneration in an open system (e.g., perifusion system). (B) Mitochondrial ATP-regenerating system plus extramitochondrial ATP-utilizing system (e.g., hexokinase + glucose: hexokinase system). (C) Extramitochondrial ATP-utilizing system plus competing mitochondrial and extramitochondrial ATP-regenerating systems (e.g., pyruvate kinase + phospho*enol*pyruvate: pyruvate kinase system). (Upper panels) (I) Oxidative phosphorylation, (II) hexokinas, (III) pyruvate kinase; (1) inhibitor, e.g., carboxyatractyloside, (T) ATP, (D) ADP; straight arrows mark flux directions. (Lower panels) The predicted response of respiration rate $d[O_2]/dt$ to small additions of carboxyatractyloside.

Materials and Methods

Chemicals and enzymes. Yeast hexokinase (EC 2.7.1.1), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), lactate dehydrogenase (EC 1.1.1.27), adenylate kinase (EC 2.7.4.3), pyruvate kinase (EC 2.7.1.40), phospho*enol*pyruvate, carboxyatractylcoside, ATP, ADP, succinate, Tris, $NADP^+$, NADH and CCCP were purchased from Boehringer Mannheim, rotenone from Sigma, St. Louis. All other chemicals were produced in analytical grade in the G.D.R.

Isolation of mitochondria. Rat liver mitochondria were isolated as previously described [20] in 0.25 M sucrose adjusted to pH 7.4 with small amounts of Tris.

Incubation conditions. The standard medium for incubation contained 110 mM sucrose, 60 mM Tris, 60 mM KCl, 15 mM glucose, 10 mM K_2HPO_4, 5 mM $MgCl_2$ and 0.5 mM EDTA. The pH was adjusted to 7.4 with HCl. The medium was completed immediately before measurements by the addition of 1 μM rotenone, 10 mM phospho*enol*pyruvate, 10 mM succinate and 1 or 2 mM ATP. The temperature was 25°C. The respiration rates and the first derivative were measured with Clark-type oxygen electrodes in a thermostatically controlled closed vessel.

Determination of control strength. Control strength (Z) for the enzymes hexokinase and pyruvate kinase were calculated from experimental

data using the equation [15]:

$$Z = \left(\frac{dF}{dE}\right)\left(\frac{E}{F}\right) \quad (1)$$

where F is the mitochondrial rate of respiration under steady-state conditions and E the activity of enzymes added. Changes of respiration caused by increasing addition of enzyme allowed the determination of control strength between the resting and active state in the same experiment. The control strength of the adenine nucleotide translocator was calculated from data obtained by titration experiments with its irreversible inhibitor carboxyatractyloside

$$Z = \left(\frac{dF}{dI}\right)\left(\frac{I_{max}}{F_0}\right) \quad (2)$$

where F_0 is the flux in the absence of the inhibitor and I_{max} is the amount of inhibitor necessary for total inhibition [17].

Assays. Mitochondrial protein was determined by the biuret method [21]. Activities of hexokinase and pyruvate kinase were measured by standard procedures [22]. For determination of adenine nucleotides, samples were withdrawn and quenched with $HClO_4$. After immediate neutralization with K_2CO_3 the samples were stored in liquid nitrogen until measurement as described previously [9].

Computer simulation. The mathematical model for the description of mitochondrial oxidative phosphorylation was identical with that described in Ref. 16. Due to the slightly different incubation conditions used here, some parameter values were changed: maximum velocities (in nmol/min per mg) of hydrogen supply $V_h = 280$, reversible electron flux $V_r = 3000$, cytochrome oxidase $V_o = 8 \cdot 10^5$, ATP synthesis $V_a = 3000$ and external ATP splitting $V_e = 60$; rate constants (in nmol/min per mg) of proton leak $k_l = 0.02$, internal ATP utilization $k_i = 0$ and external phosphate $[P_i]_E = 10$ mM. For hexokinase (v_{hk}) and pyruvate kinase (v_{pk}) the following rate equations were used:

$$v_{hk} = V_{hk}\frac{[ATP]_E}{K_{hk} + [ATP]_E} \quad (3)$$

$$v_{pk} = V_{pk}\frac{[ADP]_E}{K_{pk} + [ADP]_E} \quad (4)$$

Other terms in the complete rate laws of yeast hexokinase (e.g., see Refs. 23 and 24) and of pyruvate kinase from rabbit muscle (e.g., see Ref. 25) could be neglected under our conditions. Particularly, this concerns the inhibition of hexokinase by ADP and AMP as well as the inhibition of pyruvate kinase by ATP, the latter being very small because of the large excess of phospho*enol*-pyruvate [25]. The concentrations $[ATP]_E$ and $[ADP]_E$ were computed from the ratio $[ATP]_E/[ADP]_E = Q_E$ occurring in the model of oxidative phosphorylation [16]:

$$[ATP]_E = A_E/\left(1 + 1/Q_E + K_{eq}^{ak}/Q_E^2\right) \quad (5)$$

$$[ADP]_E = [ATP]_E/Q_E \quad (6)$$

where $A_E = [ATP]_E + [ADP]_E + [AMP]_E$ is the constant sum of external adenine nucleotides and $K_{eq}^{ak} = [ATP]_E[AMP]_E/[ADP]_E^2$ the equilibrium constant of the adenylate kinase reaction. Besides the hexokinase also the splitting of external ATP by an ATPase activity in the mitochondrial preparation was taken into account [16]. The equation was improved by introducing a Michaelis constant K_e.

$$v_e = V_e\frac{[ATP]_E}{K_e + [ATP]_E} \quad (7)$$

For the additional parameters in Eqns. 3–7 the following values were used: $K_{hk} = 0.15$ mM [26], $K_{pk} = 0.2$ mM [27], $K_{eq}^{ak} = 0.5$ [28] and $K_e = 0.15$ mM (Küster, U., personal communication). The values given to V_{hk} and V_{pk} depended on the system simulated: $V_{hk} = V_{pk} = 0$ for the perifusion system (Fig. 1A); $V_{pk} = 0$ and V_{hk} varied in the hexokinase system (Fig. 1B); $V_{hk} = 500$ nmol/min per mg and V_{pk} varied in the pyruvate kinase system (Fig. 1C).

Results

It was predicted already [3] that a partial inhibition of the adenine nucleotide translocation by carboxyatractyloside should produce different effects with respect to respiration rate in the hexokinase system compared with the more complex pyruvate kinase system. To test this prediction the control strength of the adenine nucleotide

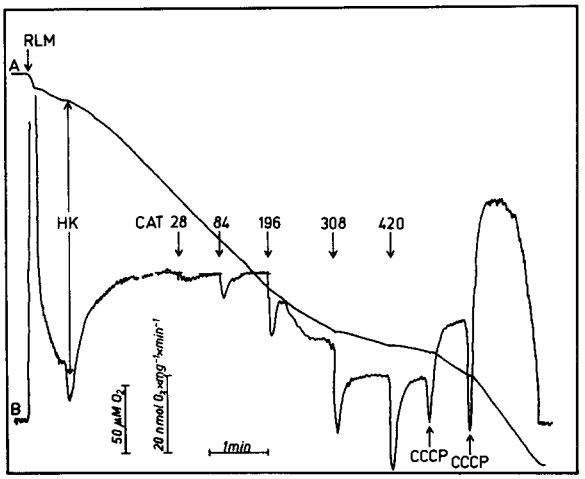

Fig. 2. Effect of carboxyatractyloside on the respiration rate in the hexokinase system (corresponding to Fig. 1B). Incubation of rat liver mitochondria (RLM) (2.1 mg protein/ml) in standard medium with 10 mM succinate, 1 μM rotenone, 1 mM ATP and 10 mM phospho*enol*pyruvate. A stationary respiration rate of 40 nmol O_2/mg protein per min was adjusted by addition of 105 mU hexokinase/mg protein (HK). Then carboxyatractyloside (CAT) was added in increasing amounts as indicated (values given in pmol carboxyatractyloside/mg protein). Rates of respiration in the first derivative (B) of the oxygen electrode signal (A). For comparison the respiration was uncoupled by additions of 5 μM CCCP.

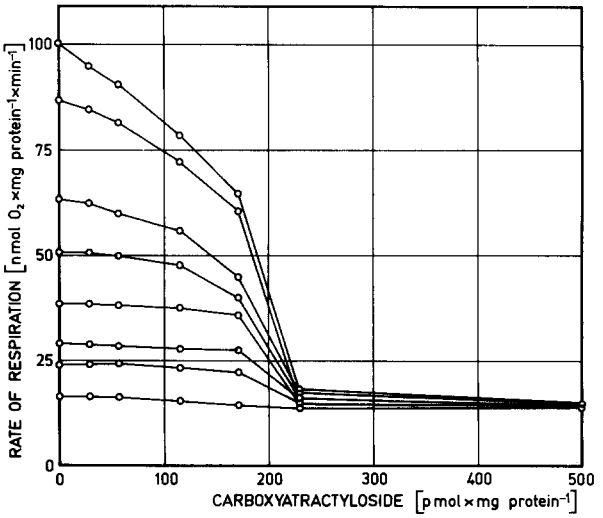

Fig. 3. Effect of carboxyatractyloside on different respiration rates in the hexokinase system (corresponding to Fig. 1B). Mitochondria (1.0 mg protein/ml) were incubated as described in Fig. 2. Different stationary states of respiration were adjusted by addition of appropriate activities of hexokinase. Slopes of the curves at zero concentration of carboxyatractyloside were used for calculation of the control strength of the adenine nucleotide translocator and enhancements in mitochondrial respiration caused by additions of hexokinase were used for calculation of the control strength of hexokinase on mitochondrial respiration. These values are presented in Fig. 4 for comparison with computed values.

translocator as well as that of the hexokinase and pyruvate kinase were determined experimentally and calculated by a mathematical model simulating the overall respiration rates in the different systems under well defined conditions. The general approach for titration experiments with carboxyatractyloside is illustrated in Fig. 2. In this experiment mitochondrial respiration was adjusted to 40 nmol O_2/mg protein per min by the addition of 105 mU hexokinase/mg protein. This corresponds to about 38% of the fully active state. The influence of increasing amounts of carboxyatractyloside on respiration rate is especially visible in the first derivative of the oxygen electrode signal. Immediately after addition of small amounts of carboxyatractyloside up to 84 pmol/mg protein, there are initial phases of inhibition, followed by a reactivation of respiration rate to a new steady state which is comparable with the original rate. High amounts of carboxyatractyloside, however, cause a remarkable decrease in respiration rate as pointed out previously [4]. The prompt response after the addition of uncoupler demonstrates the ability of the monitoring system to detect rapid changes in the respiration rate.

In Fig. 3 the results of the whole set of titration experiments with carboxyatractyloside in the hexokinase system are summarized. The respiration rates were adjusted between the resting and fully active states by addition of appropriate activities of yeast hexokinase. Obviously, the addition of small amounts of carboxyatractyloside does not influence the rate of respiration in the new steady state up to about 50% of the maximum rate. All curves have a pronounced sigmoidal shape with increasing slopes at higher concentrations of inhibitor. From the initial slope of different curves in Fig. 3 at zero concentration of carboxyatractyloside, the control strength of the adenine nucleotide translocator on mitochondrial respiration was calculated. These values are presented in Fig. 4. Additionally, the control strength of the hexokinase was calculated from differences in

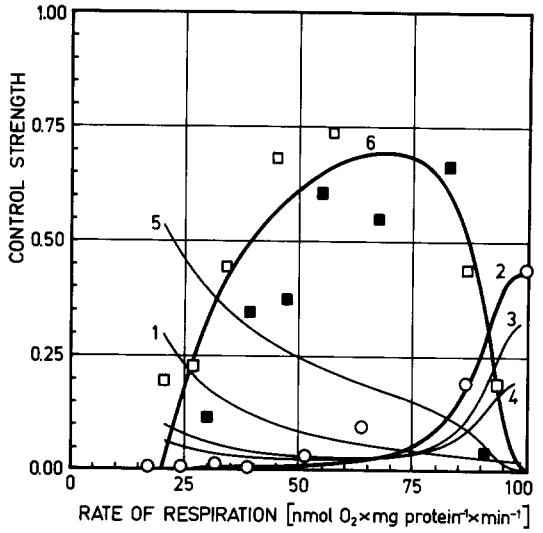

Fig. 4. Control strength of various steps in the hexokinase system (Fig. 1B) as a function of the rate of respiration calculated by computer simulation and from experimental data. Computed curves: (1) proton leak, (2) adenine nucleotide translocator, (3) hydrogen supply, (4) cytochrome oxidase, (5) extramitochondrial ATPase, (6) hexokinase. Experimental points: (○) adenine nucleotide translocator, (□,■) hexokinase. Open symbols are taken from the experiment in Fig. 3, the closed symbols from that in Fig. 5.

hexokinase activities and corresponding changes in respiration rate of experiments shown in Figs. 3 and 5. These values are shown in Fig. 4 together with curves of the control strength obtained by computer simulation for the most important steps controlling the respiration rate in the hexokinase system (Fig. 1B). Similar to the experimentally determined results, the computer simulation reflects that the adenine nucleotide translocator has practically no control strength on the steady-state respiration up to rates of about 50 nmol O_2/mg protein per min. From this rate up to that of the fully active state, the control strength of the adenine nucleotide translocator increases having the highest value at the maximum rate of coupled respiration. Under the chosen conditions, the adenine nucleotide translocator is the most important rate-controlling step in the fully active state of respiration. In the intermediate states, however, between about 30 and 80 nmol O_2/mg protein per min hexokinase has the highest control strength. For comparison, the other control strengths available from the model are also presented. Near to the resting state, the proton leak and the ATP-splitting activity in mitochondrial preparation, together causing the respiration in the resting state, possess the strongest influence on the respiration control. In the fully active state, the hydrogen supply and the cytochrome oxidase contribute to the control besides the adenine nucleotide translocator.

Fig. 5 demonstrates the effect on steady-state respiration of pyruvate kinase additions to mitochondria in the fully active state adjusted by hexokinase in the presence of glucose and phospho*enol*pyruvate. Decreasing stationary respiration rates were obtained after additions of pyruvate kinase. As demonstrated elsewhere [19], pyruvate kinase competes for ADP with the mitochondrial oxidative phosphorylation without any influence on the rate of glucose 6-phosphate formation. Considering the decrease in respiration rate after additions of pyruvate kinase, it follows that pyruvate kinase has a negative control strength on the overall reaction of mitochondrial respira-

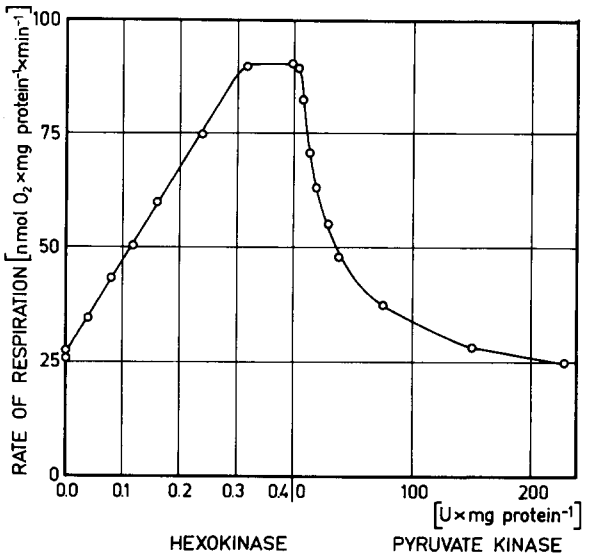

Fig. 5. Dependence of mitochondrial respiration on hexokinase activity up to the fully active state followed by additions of pyruvate kinase. Mitochondria (1.1 mg protein/ml) were incubated as described in Fig. 2. Stationary rates of respiration were adjusted by increasing additions of hexokinase up to the maximum rate. Then the sample was titrated with pyruvate kinase. Changes in respiration rates caused by enzyme additions were used for calculation of control strength and are presented in Figs. 4 and 7.

tion. Because of the unfavourable K_M^{ADP} for pyruvate kinase of 0.2 mM [27], very high activities of the enzyme are necessary for the inhibition of respiration. In this pyruvate kinase system the control strength of the adenine nucleotide translocator was determined in a way similar to that described above for the hexokinase system. The results of these experiments are depicted in Fig. 6. Stationary states of respiration without inhibitor were adjusted by addition of 295 or 354 mU hexokinase/mg protein per min and appropriate activities of pyruvate kinase. The higher hexokinase activity was used in the experiments depicted in the upper two curves of Fig. 6 because of the necessity of maximal stimulation of mitochondrial respiration. It was checked that changes of hexokinase activity in this range did not cause a significant alteration in the shape of the titration curves. It is evident that already small additions of inhibitor cause an inhibition of the respiration rate. The plot of dependency on carboxyatractyloside of the respiration rates resembles straight lines more than sigmoidal curves (cf. Fig. 3). In other words, the effect of carboxyatractyloside on the respiration rate is increased, in particular, in the slower range of respiration. This corresponds to a higher control strength of the adenine nucleotide translocator in this range compared with the hexokinase system.

The control strength calculated from Figs. 5 and 6 is compared with model calculations in Fig. 7. Indeed, the course of changes in the control strength of adenine nucleotide translocator in the pyruvate kinase system is quite different from that in the hexokinase system. It begins at low values of respiration with a maximum at about 30 nmol O_2/mg protein per min. Only at the fully active state is the control strength of 0.45 similar to that in the hexokinase system. At all other rates of respiration the control strength of translocator in the hexokinase system is much lower. On the other hand, the computed control strengths of the enzyme hexokinase within the complete systems B and C (Fig. 1), respectively, are not very different from each other (Figs. 4 and 7). A significant control strength must be ascribed to the hexokinase reation in the intermediary states of respiration which decreases to zero when approaching the

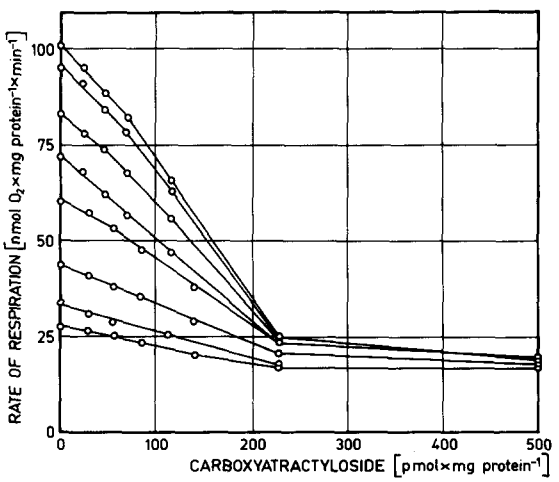

Fig. 6. Effect of carboxyatractyloside on different respiration rates in the pyruvate kinase system (Fig. 1C). For the six experiments in the lower part of the figure stationary states of respiration were adjusted by addition of 295 mU hexokinase/mg protein and appropriate activities of pyruvate kinase. In the other two experiments 354 mU hexokinase/mg protein was used. Slopes of curves at zero concentration of carboxyatractyloside were used for calculation of control strength and presented in Fig. 7.

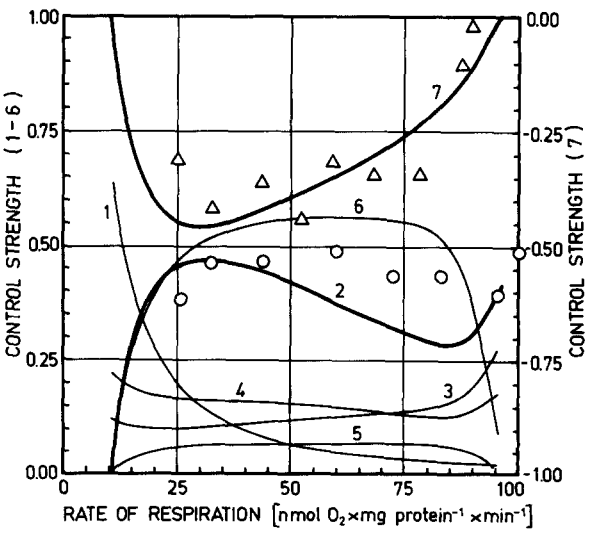

Fig. 7. Control strength of various steps in the pyruvate kinase system (Fig. 1C) as a function of the rate of respiration calculated by computer simulation and from experimental data. Pyruvate kinase [computed (7) and experimentally determined (△)] has a negative control strength (right-hand ordinate). Other symbols as in Fig. 4. The experimental points are taken from Figs. 5 and 6.

active state of respiration. In the pyruvate kinase system the sum of control strengths of hexokinase plus adenine nucleotide translocator exceeds unity because of the negative control strength of pyruvate kinase. In absolute sense, the highest degree of control strength of pyruvate kinase was estimated at rates of about 30–35 nmol O_2/mg protein per min. The control strength of pyruvate kinase decreases to zero, approaching both the level of the active state and the resting state of respiration.

The computed curves in Fig. 7 are very similar to those in Fig. 8 which were obtained for respiration control under conditions of different, but stationary, extramitochondrial adenine nucleotide patterns. Only at respiration rates near to that of the fully active state do some deviations occur. For the sake of completeness, in Fig. 8 the curves are also given for the reversible electron flux in the respiration chain and for the mitochondrial ATPase. Both reactions have a very small influence so that these curves were omitted from Figs. 4 and 7.

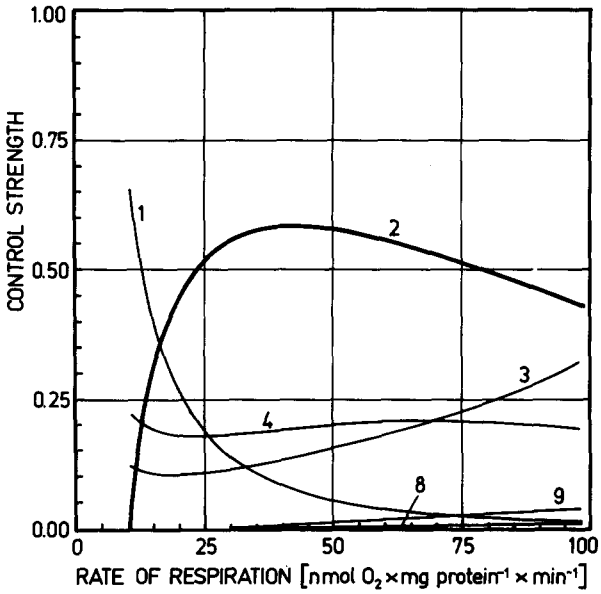

Fig. 8. Control strength of various steps in the perifusion system (Fig. 1A) as a function of the rate of respiration calculated by computer simulation. (8) Reversible electron flux through the respiratory chain, (9) mitochondrial ATPase. Other symbols as in Fig. 4.

TABLE I

CONTROL STRENGTH OF ADENINE NUCLEOTIDE TRANSLOCATOR, HEXOKINASE AND PYRUVATE KINASE ON MITOCHONDRIAL RESPIRATION IN SYSTEMS OF DIFFERENT COMPLEXITY

System A, B and C as described in Fig. 1. Control strengths of the enzymes regarded were determined experimentally for the rates of respiration (nmol O_2/mg protein per min) as well as computed (*) by means of computer simulation.

System	Adenine nucleotide translocator		Hexokinase		Pyruvate kinase	
	Rate of respiration	Control strength	Rate of respiration	Control strength	Rate of respiration	Control strength
A	25 *	0.52 *	–	–	–	–
	50 *	0.57 *	–	–	–	–
	97 *	0.43 *	–	–	–	–
B	25 *	0.00 *	23 *	0.13 *	–	–
	25	0.00	23	0.27		
	50 *	0.01 *	55 *	0.65 *	–	–
	50	0.03	55	0.60		
	100 *	0.44 *	93 *	0.20 *	–	–
	100	0.44	93	0.19		
C	26 *	0.46 *	23 *	0.44 *	25 *	−0.45 *
	26	0.37			25	−0.31
	44 *	0.44 *	55 *	0.56 *	52 *	−0.38 *
	44	0.47			52	−0.44
	96 *	0.40 *	93 *	0.23 *	90 *	−0.09 *
	96	0.39			90	−0.01

TABLE II

EFFECT OF CARBOXYATRACTYLOSIDE ON RESPIRATORY RATES AND ON ATP/ADP RATIOS IN THE HEXOKINASE (FIG. 1B) AND PYRUVATE KINASE SYSTEMS (FIG. 1C), RESPECTIVELY

Mitochondria (0.54–0.68 mg protein/ml) were incubated as described in Fig. 2 but with 2 mM ATP. Respiration rates were adjusted by addition of 81–122 mU hexokinase/mg protein for experiments with the hexokinase system or by addition of 234–258 mU hexokinase/mg protein plus 3.6–4.8 U pyruvate kinase/mg protein for corresponding experiments with the pyruvate kinase sytem. Before and after addition of 55 pmol carboxyatractyloside/mg protein, samples were withdrawn for determination of ATP/ADP ratios. CAT, carboxyatractyloside.

System	Expt. No.	Rate of respiration (nmol O_2/mg protein per min)			[ATP]/[ADP]		
		−CAT	+CAT	Δ	−CAT	+CAT	Δ
(B) Hexokinase	1	51.3	51.3	0	40.2	31.1	9.1
	2	47.0	47.0	0	40.8	32.9	7.9
	3	48.7	48.3	0.4	43.3	28.7	14.6
(C) Hexokinase + pyruvate kinase	1	46.0	40.9	5.1	44.8	43.9	0.9
	2	48.7	41.1	7.6	48.8	47.2	1.6
	3	47.4	42.0	5.4	51.9	50.9	1.0

Table I summarizes the results on control strength of the adenine nucleotide translocator, hexokinase and pyruvate kinase reactions, respectively, in the three systems compared here. The computed data were taken from Figs. 4, 7 and 8 at rates of respiration identical to those for the experimentally determined values. In the resting and intermediate states, respectively, the translocator has a remarkable control strength only in systems A and C. In the fully active state the control strength of the translocator is comparable in all three systems. On the other hand, the course of control strength of hexokinase is similar in systems B and C, occupying more than 0.5 in the intermediate states but decreasing strongly in the fully active state.

The lack of effect of small additions of carboxyatractyloside in the hexokinase system on mitochondrial steady-state respiration was explained to be caused by a decrease in the extramitochondrial ATP/ADP ratio compensating the effect of partial inhibition of the translocator [4]. Further, it was predicted that in the pyruvate kinase system no drastic changes of the ATP/ADP ratio should occur [3]. Therefore, in parallel experiments the effect of small amounts of carboxyatractyloside on the rate of mitochondrial steady-state respiration was compared with the effect on the corresponding ATP/ADP ratios in both the hexokinase and pyruvate kinase systems (Table II). Before and after additions of 55 pmol carboxyatractyloside/mg protein, samples were taken and the ATP/ADP ratios were determined. It is evident that this amount of inhibitor was small enough to have no effect on the rate of steady-state respiration in the hexokinase system whereas the ATP/ADP ratios decrease, compensating the partial inhibition of translocator. In contrast, in the pyruvate kinase system the ATP/ADP ratio practically does not change after addition of carboxyatractyloside whereas the rate of respiration was decreased distinctly.

Discussion

The results described here clearly demonstrate that it is not justified to assume the existence of one rate-controlling step in respiration. The concept of control strength shows that all enzymes of a system have some contribution to the control of metabolic fluxes [14,15]. Originally, the concept of control strength was used for analyzing linear enzyme chains, for instance, in glycolysis [15]. In this paper, the control strength of three enzymes (adenine nucleotide translocator, hexokinase and pyruvate kinase) on the respiration rate was

analyzed in a branched system. These three enzymes are constituents of the subsystems of mitochondrial ATP generation, extramitochondrial ATP generation and extramitochondrial ATP consumption, but the respiration rate only determines the flux through the mitochondrial subsystem. Similarly it should be possible to analyze the control strength on the flux through the second ATP-generating system (pyruvate kinase) or through the hexokinase. We have chosen the respiration rate since it is an indicator of mitochondrial function.

We have shown for the adenine nucleotide translocator that the degree of control exerted on the steady-state rate of respiration not only depends on the flux through the respiratory chain [4] but also on the way in which the flux is caused [3]. In the presence of hexokinase and glucose, we found a very small effect of the translocator on the stationary rate of respiration provided mitochondrial respiration was not fully activated by excess hexokinase. This result is quite different from the situation in the perifusion system where the extramitochondrial adenine nucleotide pattern is constant. In the latter case, only mitochondrial processes determine the rate of respiration and in the range of ATP/ADP-dependent respiration the translocator has the strongest influence of all steps. The changes produced by the inclusion of hexokinase are primarily caused by the fact that the extramitochondrial ATP/ADP ratio now results from both activities, ATP production by mitochondria and its consumption by the hexokinase. In the steady state, both fluxes are equal and an inhibition of adenine nucleotide translocator leads to a decrease in the ATP/ADP ratio which restores the original flux.

The strong suppression of the controlling influence of the translocator by the hexokinase is caused by the different sensitivities to changed ATP/ADP ratios. Yeast hexokinase has a low K_m value of 0.15 mM ATP [26] whereas the sensitivity to ADP inhibition is very low (K_i = 2–3 mM ADP [23,24]). In the region where mitochondrial respiration is sensitive to the ATP/ADP ratio (ATP/ADP > 5 [16]) the activity of hexokinase is, therefore, nearly independent of changes in the ATP/ADP ratio.

A much higher control strength of the translocator in the presence of hexokinase was reported by Groen et al. [17]. They found a control strength of about 0.3 at respiratory rates lower than 50% and they observed the maximum at about 65% of the active respiration. The reason is probably the unfavourable high concentration of carboxyatractyloside used by Groen et al. for the first experimental points in the inhibitor titrations resulting in overestimations of control strength especially in the range up to 70 nmol O_2/mg protein per min.

The further supplementation of the reaction system by pyruvate kinase as an additional source of ATP again restores the controlling influence of the translocator. The reason is that now the ATP/ADP ratio is mainly determined by the activities of extramitochondrial processes, i.e., hexokinase and pyruvate kinase. Changes in the activity of the translocator have much smaller effects on the ATP/ADP ratio than in the absence of pyruvate kinase and, therefore, they do not produce a compensating shift in the adenine nucleotide pattern.

In general, this interpretation of our experimental results is in agreement with the connectivity theorem of Kacser and Burns [14] connecting control strengths with elasticities (or effector strengths [15], i.e., the effects of changes in the metabolite concentration on the reaction rates). It follows from this theorem that in the absence of pyruvate kinase the ratio of the control strengths of the translocator to the hexokinase is proportional to the reciprocal ratio of their elasticities for the extramitochondrial ATP/ADP ratio. In the presence of pyruvate kinase, an additional term occurs, increasing the ratio of the control strengths proportional to the negative control strength of the added pyruvate kinase. However, for the quantitative evaluation of the agreement between experiment and theory computer simulation was necessary. The computer model is a mathematical expression of the relations considered before. Both the experimental results as well as the computer simulation clearly demonstrate that the same molecular event (inhibition of the adenine nucleotide translocator by carboxyatractyloside) produces different results in systems differing in the composition of ATP- and ADP-converting enzymes.

As shown by experimentally supported com-

puter simulations [16] and by experiments of Groen et al. [17], there are steps within the overall reaction of mitochondrial oxidative phosphorylation with negligible contribution to the control of respiratory rate. In order to obtain reconstituted systems which allow simulation of distinct situations of energy metabolism in intact cells, it is necessary to determine the really important steps. For example, the pyruvate kinase system, in addition to the hexokinase system as used in this work, exhibits properties which we should expect in cell types with high rates of glycolysis [18]. In this case the respiration is mainly controlled by mitochondrial processes, predominantly by the translocator, and partial inhibition of the translocator has strong effects on the respiration rate. Such inhibition, however, has little influence on the total energy metabolism represented by the total turnover of adenine nucleotides, since the system is able to maintain the extramitochondrial ATP/ADP ratio occurring before inhibition.

By decreasing the relative activity of pyruvate kinase a model for aerobic cells is obtained, where the glycolytic pathway is insufficient to substitute oxidative generation of ATP. If, in the control range of mitochondrial respiration, the ATP-utilizing processes are insensitive to changes in the adenine nucleotide pattern, their activities solely control the respiration. In this case an inhibition of adenine nucleotide translocation exerts a small control on the respiration rate. The ATP/ADP ratio decreases drastically, causing the compensating effect found in the hexokinase system [4]. A similar system seems to be realized in hepatocytes in which the influence of the translocator on the cellular respiration was attempted to be evaluated by Stubbs et al. [12] and Groen et al. [29]. Despite the controversal interpretations of the results obtained by these authors, in both cases a parallel decrease in the rate of respiration and gluconeogenesis was found. The latter is the main process of ATP utilization under the conditions applied. The parallel inhibition of respiration and gluconeogenesis should indicate that gluconeogenesis is sensitive to changes in the adenine nucleotide pattern in a way similar to that of respiration. Then both processes, adenine nucleotide translocation and ATP utilization, mainly should share the control. Indeed, it was found that the ATP/ADP ratio decreases also in response to the inhibition of the translocator [12].

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