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CONTROL OF OXIDATIVE PHOSPHORYLATION IN RAT HEART MITOCHONDRIA

THE ROLE OF THE ADENINE NUCLEOTIDE CARRIER

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1. Inhibitor titration experiments carried out with carboxyatractyloside, oligomycin and rotenone show that in the case of heart mitochondria the membrane-bound ATPase and the respiratory chain are the major factors controlling the rate of oxidative phosphorylation whereas the adenine nucleotide carrier exhibits no control strength. 2. As shown by carboxyatractyloside titration curves under different conditions, the relative importance of the adenine nucleotide carrier depends on the mode of regeneration (F_1-ATPase or glucose plus hexokinase) of ADP from ATP exported outside mitochondria, on the total concentration of adenine nucleotides present in the medium and on the mode of limitation of the rate of respiration (cyanide, rotenone, oligomycin or mersalyl). 3. Concomitantly with the inhibition of O_2 consumption, carboxyatractyloside brings about a rise in membrane potential. The inverse relationship between the two processes is observed for carboxyatractyloside concentrations ranging between 0.7 and 1.5 nmol per mg protein. Carboxyatractyloside concentrations below and above this range increase the membrane potential without affecting significantly the rate of respiration. 4. Titration experiments aimed at comparing the effects of ADP, carboxyatractyloside and the uncoupler, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, corroborate the conclusion that in heart mitochondria a major limiting factor in oxidative phosphorylation is the capacity of the respiratory chain.

Introduction

In mammalian cells the majority of ATP is produced by oxidative phosphorylation in the intramitochondrial space whereas ATP is utilized mainly in the cytoplasm. These opposing processes are linked via the adenine nucleotide carrier residing in the internal membrane of mitochondria and exchanging external ADP for internal ATP (for review see Ref. 1). The problem of whether or not the adenine nucleotide carrier contributes to the control of the rate of oxidative phosphorylation has been a matter of intensive discussions in the last years (for review, see Ref. 2). Applying the control theory of Kacser and Burns [3] and Heinrich and Rapoport [4], careful titrations have been carried out in isolated mitochondria with the quasi-irreversible inhibitor carboxyatractyloside [5–7] or with the reversible inhibitor atractyloside [8,9]. On the basis of these experiments, it is now

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Abbreviations: Mops, 4-morpholinopropanesulphonic acid; TPP^+, tetraphenylphosphonium; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

generally accepted that the adenine nucleotide carrier exerts a partial control over the rate of oxidative phosphorylation. The control strength of the adenine nucleotide carrier is, however, not constant, but depends on various factors like the rate of respiration [6,7], the total concentration of adenine nucleotides [10] or the presence of extramitochondrial ADP-consuming processes [7]. The situation is probably similar in intact cells as it was shown that atractylosides inhibit both the respiration of hepatocytes [11] and different metabolic processes dependent on extramitochondrial ATP [12,13].

The adenine nucleotide carrier is certainly not the only regulatory factor as the substrate supply of the respiratory chain and the cytochrome oxidase activity were shown to exert an important control strength as well [6,7]. On the other hand, small quantities of oligomycin or antimycin A do not influence the rate of respiration [14] indicating that neither the ATPase nor the *bc*-region of the respiratory chain represent significant controlling factors.

All the above data were obtained with rat liver mitochondria or rat liver cells. The possibility of tissue differences was not raised in spite of known variations in the enzyme contents [15–18]. The aim of the work reported in the present paper was to investigate the factors controlling oxidative phosphorylation in rat heart mitochondria and to compare the role of the adenine nucleotide carrier under different experimental conditions.

Materials and Methods

Biological preparations

Rat heart mitochondria were prepared according to Ref. 19. The isolation medium consisted of 0.075 M sucrose, 0.225 M mannitol, 0.5 mM EDTA and 5 mM Mops, the pH being 7.3. The protein content was determined by the biuret method using bovine serum albumin as control. F_1-ATPase was prepared from bovine heart mitochondria according to Ref. 20; its specific activity was between 70 and 90 μmol ATP hydrolyzed per min per mg at 25 °C.

Measurement of respiration and membrane potential

O_2 consumption was measured polarographically with a Clark electrode (Yellow Springs, Cleveland). O_2 consumption and membrane potential were measured concomitantly, under identical conditions. The medium consisted of 120 mM KCl, 5 mM $MgSO_4$ and 10 mM potassium phosphate (pH 7.3) supplemented with 5 μM tetraphenylphosphonium (TPP^+) for the potential measurements. The final volume was 1.4 ml, and 7 mM pyruvate plus 0.7 mM malate were added as respiratory substrates. The temperature was set at 25 °C. Mitochondrial membrane potential was calculated from the accumulation ratio of the lipophilic cation TPP^+ as described by Kamo et al. [21]. Membrane potential was derived from the equation:

$$\Delta\psi = 2.3\frac{RT}{F}\log\frac{v}{V} - 2.3\frac{RT}{F}\log(10^{F\Delta E/2.3RT} - 1)$$

where v is the volume of the matrix space of mitochondria, V the external volume, and ΔE the variation in the electrode potential [21]. The uptake of TPP^+ was corrected for TPP^+ binding in the presence of the uncoupler FCCP. The volume of the matrix space was derived from the difference between the volumes occupied by ^3H_2O (total space) and [^{14}C]sucrose (external space plus intermembrane space); the average value found in five determinations was 0.7 μl per mg protein. It was checked that carboxyatractyloside at the concentrations used does not modify the volume of the matrix space. The extramitochondrial concentration of TPP^+ was followed by an ion selective electrode attached to a Radiometer 25 pH meter and a Sefram Type PE potentiometric recorder. In all traces, specific binding of TPP^+ was assessed by addition at the end of the assay of either the uncoupler carbonylcyanide *p*-trifluoromethoxyphenylhydrazone or the respiratory inhibitor KCN; in both cases, similar values were obtained.

Measurement of control strength

The response of a pathway flux to different concentrations of an inhibitor I on a given enzyme of this pathway is described by an inhibition curve which can be used for calculation of the control strength of the enzyme [3]. This method has been used to determine the control strength of the

adenine nucleotide carrier [5]. The initial slope of the inhibition curve dJ/dI, i.e., the decrease in flux through the pathway for small increments of inhibitor is related to the control strength, C, by the equation:

$$C = \frac{dJ/J}{dI/I}$$

where J is the flux in the absence of inhibitor and I the concentration of inhibitor required for full inhibition. The above equation applies to the so-called irreversible inhibitors, i.e., to inhibitors that bind to receptor sites with high affinity. Among the inhibitors used in the present work, carboxyatractyloside is clearly a quasi-irreversible inhibitor [22]. Active concentrations for rotenone and oligomycin are also in the nanomolar range.

The control strength of the adenine nucleotide carrier was determined under two different types of conditions, namely linear and cyclic systems of oxidative phosphorylation. A linear system of oxidative phosphorylation was obtained with respiring mitochondria in the presence of an excess of ADP (Scheme I, Fig. 1); in this case, ADP enters the mitochondria, is phosphorylated into ATP which is exported and accumulates outside. In the cyclic system, the exported ATP is dephosphorylated by added F_1 (F_1-system) (Scheme II, Fig. 1), or by hexokinase in the presence of glucose to regenerate ADP (hexokinase system). In both cases, P_i was present in large excess.

Measurement of ADP transport

The rate of [^{14}C]ADP transport was measured at 0 °C, with a saline medium made of 120 mM KCl/10 mM Tris-HCl/1 mM EDTA (final pH, 7.3; final volume, 1 ml). Mitochondria (1 mg protein) were preincubated in the reaction medium for 2 min prior to addition of [^{14}C]ADP; final concentration, 100 μM. The incubation lasted for 10 s at 0 °C; it was stopped by 4 μM carboxyatractyloside, followed by rapid centrifugation. The amount of [^{14}C]ADP accumulated into the matrix space by specific transport was calculated from the difference between the amount of [^{14}C]ADP found in the mitochondrial pellet under the previous conditions and that accumulated when mitochondria had been preincubated with 4 μM carboxyatractyloside for 2 min, followed by 100 μM [^{14}C]ADP. The rate of transport was calculated as previously described [23].

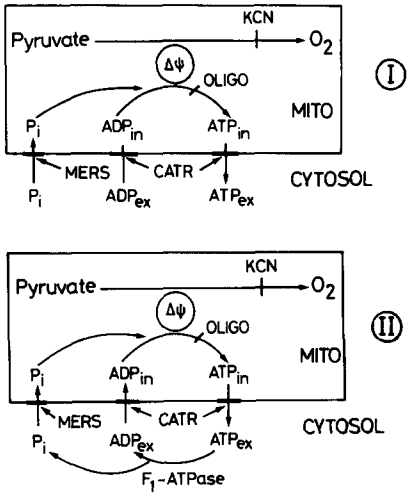

Fig. 1. Schemes illustrating the linear (I) and cyclic (II) systems of oxidative phosphorylation used in the present work. CATR = carboxyatractyloside; MERS = mersalyl; OLIGO = oligomycin.

Results

Contribution of different steps to the control of oxidative phosphorylation in heart mitochondria

The capacity of the AdN carrier, the H^+-ATPase, and the NADH dehydrogenase in the respiratory chain to control the rate of oxidative phosphorylation in heart mitochondria oxidizing pyruvate was evaluated on the basis of titrations with carboxyatractyloside, oligomycin and rotenone (Fig. 2). Carboxyatractyloside acts in less than 2 s, but the other inhibitors have to be preincubated with mitochondria for 60 s. As shown in Fig. 2A and B small quantities of both oligomycin or rotenone brought about a clear inhibition of respiration and a definite initial slope could be determined. Calculation of the control strength according to Ref. 5 gave a value of 0.46 for the ATPase and 0.37 for the respiratory chain. In contrast to this, up to 0.7 nmol carboxyatractyloside per mg protein did not decrease O_2 consumption (Fig. 2C). Complete inhibition of ADP-stimulated respiration was achieved by 1.4–1.5 nmol carboxyatractyloside per mg mitochondrial

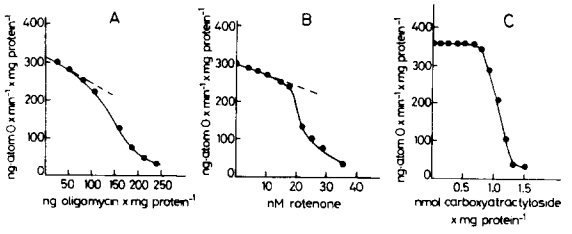

Fig. 2. Distribution of the control of oxidative phosphorylation in rat heart mitochondria. Titrations with oligomycin (A), rotenone (B) and carboxyatractyloside (C). The medium contained 0.6 mM ATP in A, B and 2.5 mM ATP in C, 9 mM glucose and 0.8 U hexokinase per mg protein. The amount of mitochondrial protein was 0.53 mg per ml.

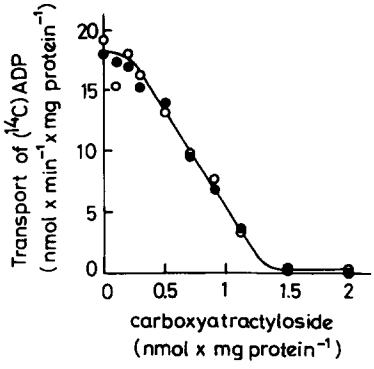

Fig. 3. Effect of increasing concentrations of carboxyatractyloside on the rate of [^{14}C]ADP transport in rat heart mitochondria. Mitochondria (1 mg protein in 1 ml medium) were preincubated for 2 min with increasing concentrations of carboxyatractyloside up to 2 nmol/mg protein. Incubation was carried out as described in Materials and Methods. Control, (○——○); oligomycin added at 150 ng per mg protein, (●——●).

protein. This value corresponds to the amount of adenine nucleotide carrier present in rat heart mitochondria [15,16]. The fact that ADP-stimulated respiration is not affected by small concentrations of carboxyatractyloside up to 0.7 nmol per mg protein suggests that under the given conditions the adenine nucleotide carrier does not contribute to the control of oxidative phosphorylation.

The sigmoidal shape of the curve of inhibition of ADP-stimulated respiration by increasing concentrations of carboxyatractyloside recalled the sigmoidal titration of ADP transport in liver mitochondria [22], although in the latter case the lag was much less pronounced. This lag effect was interpreted in terms of cooperative interactions between carboxyatractyloside binding sites [22]. We therefore set out to determine whether the carboxyatractyloside titration of ADP transport in rat heart mitochondria exhibited a lag at low concentrations of the inhibitor, and accordingly what was the size of the lag as compared to that found for the ADP-stimulated respiration. As shown in Fig. 3, there was indeed no significant inhibition of [^{14}C]ADP transport by low concentrations of carboxyatractyloside up to 0.2 nmol per mg protein; above this concentration, transport activity decreased steadily, full inhibition being attained at 1.4–1.5 nmol carboxyatractyloside per mg protein as in the case of the ADP-stimulated respiration. These data clearly show that a lag in the effect of carboxyatractyloside on ADP transport exists, but its size is 2- to 3-fold smaller than that observed for the titration of the ADP-stimulated respiration by carboxyatractyloside. Furthermore, at 0.7 nmol of carboxyatractyloside per mg protein, a concentration that does not modify significantly the rate of respiration, the rate of ADP transport was decreased by 50%. In other words, the absence of control strength at low concentration of carboxyatractyloside in rat heart mitochondria cannot be entirely attributed to the sigmoidal titration of ADP transport by carboxyatractyloside.

Control of oxidative phosphorylation by adenine nucleotide transport under various experimental conditions

The experiments of Fig. 2 were carried out in a system where a maximal rate of ADP regeneration was achieved by the addition of an excess of hexokinase. It was interesting to test whether the adenine nucleotide carrier behaves similarly under various experimental conditions. Fig. 4 shows titrations with carboxyatractyloside in a system where ADP was regenerated by isolated beef heart F_1-ATPase (Fig. 4A) and in an 'open' system (Fig. 4B), where a large amount of ADP without regenerating system was added to mitochondria. In the latter case it was verified that the applied quantity of ADP was high enough to ensure both a stable rate of respiration and a stable level of membrane potential during the entire period of the measurement. In fact, slow regeneration of ADP might

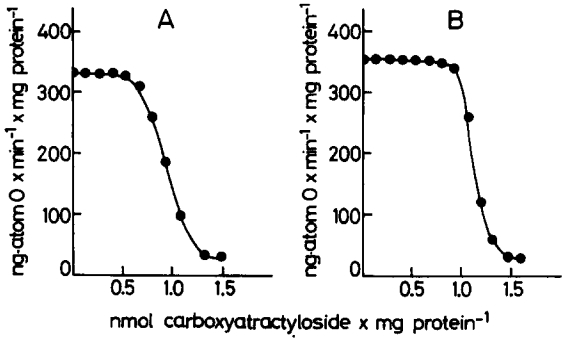

Fig. 4. Comparison of the effect of carboxyatractyloside on the rate of respiration in the F_1-system (A) and the open system (B). Respiration was stimulated by the addition of 2.5 mM ATP plus 1 μg of bovine heart mitochondrial F_1-ATPase (A) or 2.5 mM ADP (B). The amount of mitochondrial protein was 0.53 mg per ml.

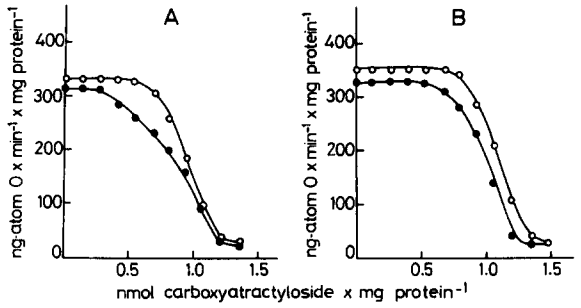

Fig. 5. Effect of adenine nucleotide on the inhibition by carboxyatractyloside of the rate of respiration in the F_1-system (A) and in the hexokinase system (B). Respiration was stimulated by 1 μg bovine heart mitochondrial F_1-ATPase (A) or by 9 mM glucose plus 1.1 U hexokinase per mg protein (B). The medium contained 0.53 mg mitochondrial protein per ml and either 2.5 mM ATP (○——○) or 0.6 mM ATP (●——●).

have occurred also in this system due to the activity of adenylate kinase. The shape of the carboxyatractyloside titration curves obtained in both of these systems was similar to that shown in Fig. 2. However, in spite of the strongly sigmoidal character, reproducible differences could be observed between the three experimental conditions. Irrespectively of the rate of maximal respiration, inhibition began in the F_1 system always at a lower concentration of carboxyatractyloside than either in the hexokinase system or in the open system. The slightest decrease of O_2 consumption was observed at 0.80 nmol carboxyatractyloside per mg in the hexokinase system (Fig. 2C), at 0.55 nmol per mg protein in the F_1-system (Fig. 4A), and only at 0.93 nmol per mg in the open system (Fig. 4B). The half inhibition of O_2 uptake was observed at 1.07, 0.95 and 1.15 nmol carboxyatractyloside per mg protein, respectively. Fig. 5 shows carboxyatractyloside titrations in the presence of two different concentrations of adenine nucleotides. In both cyclic systems, a decrease of the adenine nucleotide concentration shifted the titration curve to the left, i.e., the inhibition of respiration started with lower amounts of carboxyatractyloside. Thus, the data in Fig. 4 and 5 prove that, although the control strength of the adenine nucleotide carrier cannot be expressed in mathematical terms, its importance depends on the actual conditions of measurement. This finding fits with the statement of Kacser and Burns [3] that the control strength of an enzyme depends on the elasticity coefficients of that enzyme and the other pathway enzymes to their substrates and products.

Variations of the rate of O_2 consumption represent another point of interest, as in liver mitochondria it was found that the control strength of the adenine nucleotide carrier decreased as the rate of respiration was lowered [5,7]. In all these experiments, O_2 consumption was manipulated by addition of different amounts of hexokinase. Without doubt, variations of the rate of extramitochondrial ATP splitting simulate appropriately the physiological situation. However, in order to test whether it is only the rate of respiration that determines the control strength of the adenine nucleotide carrier, it seemed worthwhile to carry out carboxyatractyloside titrations under conditions where O_2 consumption is limited by different means. The results are summarized in Fig. 6. When the rate of respiration was gradually decreased by partial inhibition of cytochrome oxidase by cyanide (Fig. 6A), the initial plateau of the carboxyatractyloside titration curves became longer, i.e., more carboxyatractyloside could be added without diminishing O_2 consumption. Inhibition of the respiratory chain by antimycin A or rotenone or variation of the concentration of F_1 had the same effect as cyanide. It should be noticed that in the case of liver mitochondria, variations of the amount of hexokinase resulted in similar changes of the carboxyatractyloside titration curves [5,7]. In other

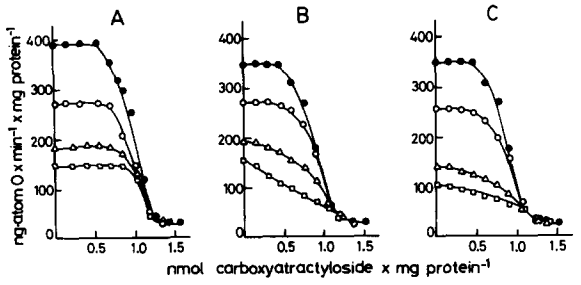

Fig. 6. Effect of carboxyatractyloside on the rate of respiration in the presence of various concentrations of cyanide (A), oligomycin (B) or mersalyl (C). The medium contained 2.5 mM ATP plus 1 μg bovine heart mitochondrial F_1-ATPase and 0.47 mg mitochondrial protein per ml. ●———●, control. Respiration was limited in A by 43 (○———○) or 89 (△———△) or 143 μM KCN (□———□); in B by 91 (○———○) or 121 (△———△) or 151 ng oligomycin (□———□) per mg protein; in C by 9.1 (○———○) or 12.1 (△———△) or 13.6 nmol mersalyl (□———□) per mg protein.

words, whatever the rate of respiration imposed by respiratory inhibitors or by the renewal of external ADP, adenine nucleotide transport is not rate limiting. The results shown in Fig. 6B and C are in sharp contrast to the previous ones. When the rate of ADP-stimulated respiration was limited by oligomycin (Fig. 6B) or by mersalyl (Fig. 6C), then a pronounced inhibition was already detected for small quantities of carboxyatractyloside, and the initial plateau of the carboxyatractyloside titration curves gradually disappeared. Thus, when respiration is limited indirectly by inhibition of the membrane-bound ATPase by means of oligomycin or by inhibition of P_i transport by means of mersalyl, the adenine nucleotide carrier becomes a significant controlling factor. The appearance of the control strength of the adenine nucleotide carrier on respiration in the presence of oligomycin is not reflected by a similar effect of oligomycin on inhibition of ADP transport by carboxyatractyloside (Fig. 3). This affords complementary evidence that the plateau observed in the titration curve of respiration by carboxyatractyloside (Fig. 2) is not attributable to the sigmoidal titration of AdN transport by carboxyatractyloside (Fig. 3).

Effect of carboxyatractyloside on the mitochondrial membrane potential

Initiation of ATP synthesis is known to decrease the H^+ electrochemical potential difference existing between the two sides of the inner mitochondrial membrane. The question arises whether the gradual decrease in the rate of ATP synthesis is also reflected by changes of the protonmotive force. In order to answer this problem, we carried out titrations with carboxyatractyloside while continuously monitoring membrane potential. Under our experimental conditions with 10 mM phosphate present in the incubation medium, the pH gradient should be minimal and the protonmotive force should be represented almost entirely by the membrane potential. This prediction has been tested in control experiments in the presence of nigericin. Due to its capacity to exchange H^+ for K^+, this antibiotic is able to collapse the pH gradient without impairing energy-conservation processes. In our system, addition of 360 ng nigericin per mg protein increased the value of the membrane potential by 5 mV, and this value was constant throughout the titrations. Thus, in the present experimental conditions, changes in protonmotive force are essentially reflected by changes in membrane potential.

The result of the titration by carboxyatractyloside is shown in Fig. 7. The decrease of the rate of respiration is accompanied by a step-by-step increase of membrane potential. However, it is interesting to note that low quantities of carboxyatractyloside (below 0.7 nmol per mg) which do not significantly influence the rate of O_2 consumption bring about a definite and very reproducible rise in potential from 180 mV to about 190 mV. Also

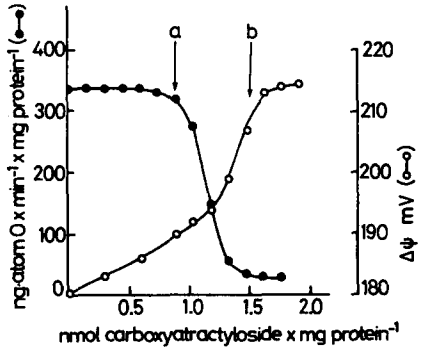

Fig. 7. Comparison of the effect of carboxyatractyloside on the rate of respiration and the membrane potential. The mitochondrial protein content was 0.48 mg per ml. Respiration was stimulated by 2.8 mM ADP. The $\Delta\psi$ is given in mV. The ΔpH was less than 5 mV.

noticeable is the small rise in potential from 208 to 215 mV which is detected at the end of the titration, when the carboxyatractyloside concentration is increased from 1.5 to 1.7 nmol per mg protein. As checked with nigericin, a possible conversion of the residual pH gradient into membrane potential was ruled out. This rise in potential is not either accompanied by a significant decrease in the rate of respiration. In brief, three regions of the carboxyatractyloside titration curves for O_2 uptake and membrane potential can be considered (Fig. 7). The first one from zero to 0.7 nmol carboxyatractyloside (arrow a) corresponds to conditions for which the adenine nucleotide carrier is apparently not rate-limiting. For carboxyatractyloside concentrations between 0.7 and 1.5 nmol per mg protein, (region between arrows a and b) adenine nucleotide transport becomes a rate-limiting reaction. At 1.5 nmol carboxyatractyloside per mg protein, state-4 is attained; yet a further addition of carboxyatractyloside up to 1.7 nmol results in a slight, but significant increase in membrane potential. It was checked that the increases in potential that are not accompanied by significant changes in the rate of respiration are not due to artifactual interactions between carboxyatractyloside and TPP^+.

More attention was paid to the region of the carboxyatractyloside titration curve where in spite of a fairly constant rate of respiration addition of carboxyatractyloside up to 0.7 nmol per mg protein results in a 10 mV increase of potential (from 180 to 190 mV). Although this increase in potential is small, it is not negligible compared to the further 20 mV increase (190–210 mV) that accompanies the 10-fold decrease in the rate of respiration (300–30 ng atom O/min per mg protein), when the carboxyatractyloside concentration is increased from 0.7 to 1.5 nmol per mg protein. This situation is surprising all the more as transport activity is reduced by 50% at 0.7 nmol carboxyatractyloside per mg protein (Fig. 3). A tentative explanation that takes into account the idea of a self regulation of adenine nucleotide transport is as follows. In the presence of excess ADP, all the adenine nucleotide carriers are actively exchanging internal ATP^{4-} for external ADP^{3-}, an electrogenic process which consumes membrane potential. Decrease of transport activity by partial inhibition of transport reduces the energy demand, and as a result membrane potential should rise. This increase in potential might in turn be partly used to force the carriers still active to work at higher rate which implies that in the absence of carboxyatractyloside and at maximal ADP load, the carriers are not working at their maximal turnover. This self-adjustment of transport activity, which consists in palliating transport deficiency caused by carboxyatractyloside inactivation of a fraction of the carriers by a higher turnover of the remaining carriers, might contribute to maintaining the rate of O_2 uptake at a fairly constant value.

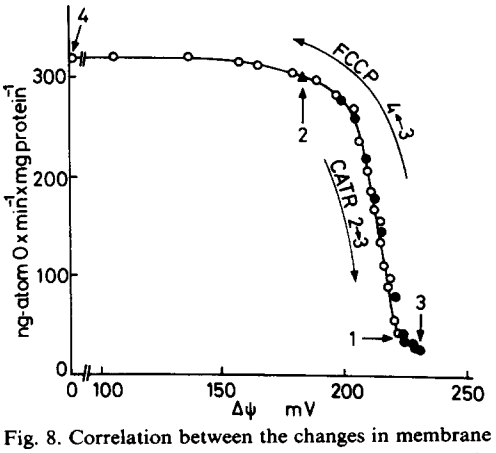

Fig. 8. Correlation between the changes in membrane potential ($\Delta\psi$) and the rate of O_2 uptake. The amount of mitochondria used was 0.50 mg/ml. Titration started with mitochondria supplemented with pyruvate plus malate. $\Delta\psi$ was 220 mV (arrow 1). ADP was added at the final concentration of 2 mM; the potential decreased to 180 mV and the respiration rate increased from 24 to 310 ng atom O/min per mg protein (arrow 2). Carboxyatractyloside (CATR) was then added in small increments (CATR 2 → 3) resulting in a step by step increase of $\Delta\psi$ up to 230 mV and in a decrease of the rate of respiration down to a value of 18 ng atom O/min per mg protein (●———●). At this point (arrow 3), FCCP was added in small increments (FCCP 3 → 4) reversing the effects of CATR on $\Delta\psi$ and on the rate of respiration (○———○), till full uncoupling was attained (arrow 4).

The highly regulated control of respiration by adenine nucleotide transport, which is postulated above, is seriously casted in doubt by the following experiment that is based on comparison of the titrations of both membrane potential and respiration by the uncoupler FCCP on one hand, and by ADP and carboxyatractyloside on the other

(Fig. 8). Membrane potential and respiration were first varied by addition of an excess of ADP; the initial potential of 220 mV dropped to about 180 mV (Fig. 8, arrow 2) like in the experiment of Fig. 7, and the rate of respiration increased from 24 to 310 ng atom O/min per mg protein. Then carboxyatractyloside was added in small increments; the value of the potential increased steadily to 230 mV. (Fig. 8, arrow 3). In accordance with the data of Fig. 7, when small increments of carboxyatractyloside were added, the effect on membrane potential relative to that on respiration was more pronounced at low potential (180–200 mV) than at higher potential (200–220 mV). The final titration was with FCCP. The potential was diminished step by step and when full uncoupling was attained, the rate of respiration levelled up to 320 ng atom O/min per mg protein. The titration curve describing the variations of potential and rates of respiration in the presence of FCCP was strictly superimposable on the titration curves obtained with ADP and carboxyatractyloside. Obviously, the titration data in the presence of FCCP cannot be explained by a self adjustment of the ADP transport activity; there is no other alternative, but to admit that under the present conditions the predominant limiting factor in the ADP-stimulated respiration is the capacity of the respiratory chain or the rate of delivery of respiratory substrates to mitochondria.

Discussion

A number of reports [5,7,14] have shown that in rat liver mitochondria the control of oxidative phosphorylation is distributed between several steps, the adenine nucleotide transport and the supply of respiratory substrates representing the most important factors in the active states whereas the ATPase and the initial segments of the respiratory chain have only minor importance. In the present paper, we show that in heart mitochondria, the situation is basically different. In heart mitochondria, the membrane-bound ATPase and the NADH dehydrogenase in the respiratory chain exert a significant controlling function, but the adenine nucleotide carrier does not exhibit any control strength over the rate of ADP-stimulated respiration. This difference between the two tissues corresponds well to the known variations in their enzyme contents. Bertina et al. [17] reported on the basis of aurovertin binding data that heart mitochondria contain about twice as much F_1-ATPase than liver mitochondria do (0.27 μmol vs. 0.12 μmol per g protein). The value found for the H^+-ATPase complex in heart mitochondria on the basis of reconstitution experiments, 0.22 μmol per g protein [18] was close to that found by Bertina et al. [17]. Based on titration data with radiolabeled atractyloside or carboxyatractyloside, the number of adenine nucleotide carrier units was approximated to 0.2–0.3 μmol per g protein in liver mitochondria [15] and 1.0–1.2 μmol per g protein in heart mitochondria [24]. Thus, the proportion of the adenine nucleotide carrier to the F_1-ATPase is approx. 2 in liver mitochondria and between 4 and 5 in heart mitochondria, explaining the difference in the distribution of the control strength. During the preparation of this manuscript, Forman and Wilson [25] reported sigmoidal carboxyatractyloside titrations in heart mitochondria. Our findings (Fig. 2C) support their results.

A comparison of the curves shown in Fig. 4 reveals that the importance of the adenine nucleotide carrier in controlling the rate of oxidative phosphorylation depends on the conditions of the incubation, for example the concentration of adenine nucleotide or the mode of regeneration of ADP. Similar results were obtained on liver mitochondria by Groen et al. [10] and Tager et al. [26]. In an intact cell, the adenine nucleotide concentration is rather stable; yet, the proportion of ATP consumption by different metabolic pathways is subject to considerable variations. Thus, in vivo the control strength of the adenine nucleotide carrier might vary even within one particular tissue.

The results presented in Fig. 6 clearly show that it is not the rate of respiration per se which influences the controlling function of the adenine nucleotide carrier; the decisive factor is the way in which O_2 consumption is limited. The control by the adenine nucleotide carrier begins at higher concentrations of carboxyatractyloside when the respiratory chain is partially inhibited; it begins at lower concentrations of carboxyatractyloside when inhibition bears on the P_i carrier or the membrane-bound ATPase. Beyond this statement

we are restricted to speculations. Phenomenologically, changes of the titration curves similar to those shown in Fig. 6B and C were observed earlier in double-inhibitor experiments [27–29] and interpreted as indications for localised interactions between the respective protein complexes. However the H^+-ATPase and the ADP/ATP carrier have different control strengths, which suggests that these enzymes are not structurally related.

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