Biochimica et Biophysica Acta, 408 (1975) 319–330

BBA 47009

RESPIRATORY CONTROL IN ISOLATED PERFUSED RAT HEART. ROLE OF THE EQUILIBRIUM RELATIONS BETWEEN THE MITOCHONDRIAL ELECTRON CARRIERS AND THE ADENYLATE SYSTEM

ILMO E. HASSINEN and KALERVO HILTUNEN

Department of Medical Biochemistry, University of Oulu, SF-90220 Oulu 22 (Finland)

(Received May 21st, 1975)

SUMMARY

The effects of KCl-induced cardiac arrest on the redox state of the fluorescent flavoproteins and nicotinamide nucleotides and on that of cytochromes *c* and *a* were studied by surface fluorometric and reflectance spectrophotometric methods. These changes were compared with measurements of the concentrations of the adenylate system, creatine phosphate, some intermediates of the tricarboxylic acid cycle and reactants of the glutamate dehydrogenase system.

KCl-induced cardiac arrest caused reduction of the fluorescent flavoproteins and nicotinamide nucleotides, oxidation of cytochromes *c* and *a*, inhibition of oxygen consumption and an increase in the $ATP/(ADP \times P_i)$ ratio. The increase in the latter was due mainly to a decrease in the concentration of P_i and an equivalent increase in creatine phosphate. The cytochromes *c* and *a* were maintained at equal redox potential and changed in parallel. When the redox state of the mitochondrial NAD couple was calculated from the glutamate dehydrogenase equilibrium, the free energy change (ΔG) corresponding to the potential difference between the NAD couple and cytochrome *c* was 115.8 kJ/mol in the beating heart and 122.2 kJ/mol in the arrested heart. The ΔG values of ATP hydrolysis calculated from the concentrations of ATP, P_i and ADP, corrected for bound ADP, were 111.1 kJ/2 mol and 115.4 kJ/2 mol in the beating and arrested heart respectively.

The accumulation of citrate and the direction of the redox changes in the respiratory carriers indicate that the tricarboxylic acid cycle flux is controlled by the respiratory chain. The data also show a near equilibrium between the electron carriers and the adenylate system and suggest that the equilibrium hypothesis of mitochondrial respiratory control is applicable to intact myocardial tissue.

INTRODUCTION

The phenomenon of respiratory control [1, 2] which can be demonstrated with isolated mitochondria is thought to be applicable also to the metabolic regulation of mitochondria in situ. However, in studies on intermediary aerobic metabolism the

role of the mitochondrial respiratory chain proper has usually been overlooked, probably owing to experimental difficulties.

In the recent times, spectrophotometric and fluorometric methods at whole-organ level have developed into powerful tools for the study of energy metabolism [3], yielding results not obtainable by standard biochemical techniques, e.g. analysis of the metabolite profiles of tissues.

To observe the respiratory control, advantage has been taken, in the present case, of the magnitude and speed of changes in the energy demand of the heart muscle elicited by changing its mechanical work [4].

Surface fluorometric and reflectance spectrophotometric methods were used to monitor the redox states of certain components of the mitochondrial respiratory chain in an isolated perfused rat heart. Changes in the redox state were correlated with changes in the adenylate system and some intermediates of the tricarboxylic acid cycle. The results demonstrated that a near-equilibrium exists between the redox carriers and the adenylate system, and the metabolite profile showed regulation above the tricarboxylic acid cycle. The drastic changes observed in the oxygen consumption of the heart in response to cardiac arrest can mostly be accounted for by the equilibrium relations in the respiratory chain.

EXPERIMENTAL

Reagents. Standard reagents were obtained from E. Merck AG, Darmstadt, Germany. Enzymes, carbonyl cyanide *m*-chlorophenylhydrazone and rotenone were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. The nucleotides were purchased from Boehringer CmbH, Mannheim, Germany and octanoic acid from Fluka AG, Buchs, Switzerland.

Rats. Female Long-Evans rats from the Department's own stocks were used. No fasting period preceded the experiments. The rats were anesthetized with intraperitoneal Nembutal and injected intravenously with 500 I.U. heparin one minute before the excision of the heart.

Heart perfusion. The hearts were perfused with Krebs-Ringer bicarbonate solution [5] containing 2.5 mM Ca^{2+} and 10 mM glucose in equilibrium with 95 % O_2/5 % CO_2, by the Langendorff procedure [6] at a hydrostatic pressure of 80 cm H_2O. Oxygen concentration in the venous perfusate was monitored by a Radiometer E5046 oxygen electrode.

Surface fluorometry. Whole-organ fluorometry was performed with a laboratory-built fluorometer [7] which was modified for heart perfusions as follows. A thermostatically-controlled chamber for the heart was constructed of Lucite and the fluorescence excitation light and fluorescence emission was led to and from the surface by means of a solid glass rod connected to a two-branch fibre optic light conductor (Schott, Mainz, Germany). The polished end of the glass rod was pressed against the heart. This arrangement was found essential to avoid artifacts arising from any change in distance between the optics and the surface of the heart. A tungsten iodide lamp was used as the light source, the primary filter being a Corning No. 5840 and the secondary filter a combination of Corning Nos 4303 and 3387 for the nicotinamide nucleotide fluorescence. For the measurement of flavin fluorescence

a Farrand 465 nm interference filter and a Corning No. 3484 filter were used as the primary and secondary filters respectively.

Reflectance spectrophotometry. Changes in the reflectance spectra were recorded by the dual wavelength method [8]. Identical optical geometry was used as in the fluorescence measurements, viz. a glass rod pressed against the surface of the heart. The measuring and reflectance light beams at wavelengths selected with a laboratory-built dual-wavelength spectrophotometer were time-shared and led through fibre optics into the glass rod. Wavelength pairs 550–540 nm and 605–630 nm with 3.3 nm spectral bandwidth were used for cytochromes *c* and *a* respectively. The steady state oxidation-reduction states of cytochromes *a* and *c* were determined by taking the rotenone-inhibited state as fully oxidized and the cyanide-inhibited state as fully reduced.

Heart extracts. Samples were obtained from the heart using aluminum clamps cooled with liquid nitrogen [9]. Initial acid extraction was performed from the frozen, pulverized sample using 8 % v/v perchloric acid in 40 % v/v ethanol, precooled to −20 °C to ensure quenching in the frozen state [10]. Extraction was repeated with 6 % v/v perchloric acid and the filtrate neutralized to pH 6 with 3.75 M K_2CO_3 containing 0.5 M triethanolamine hydrochloride.

Metabolites. The metabolite assays were performed by conventional enzymatic procedures. ADP was determined according to Adam [11]. ATP was determined using hexokinase and glucose-6-phosphate dehydrogenase [12] and phosphocreatine in the same assay by a subsequent addition of creatine kinase.

Citrate was measured with citrate lyase [13], isocitrate according to Goldberg and Passonneau [14], α-oxoglutarate according to Narins and Passonneau [15] and inorganic phosphate with phosphorylase *a* essentially according to Gawehn [16]. Glutamate was determined after Bernt and Bergmeyer [17], ammonia after Kun and Kearney [18], lactate after Hohorst [19], pyruvate after Bücher et al. [20] and glucose by a modification of the method of Hugget and Nixon [21]. All assays were completed within 24 h and the phosphate assays within 90 min after the termination of the experiment.

RESULTS AND DISCUSSION

It is known that the beating heart can be rapidly arrested by an excess of KCl [4]. In the present investigation this was done by changing the perfusion medium to a Krebs-Ringer bicarbonate solution containing 15 mM KCl. There followed a rapid inhibition of respiration (Table I).

The experiments depicted in Fig. 1. show the fluorescence changes of flavoproteins and nicotinamide nucleotides upon the addition of octanoate and subsequent redox changes resulting from the KCl-induced arrest of the heart. Initially the perfusion medium contained 10 mM glucose as the only external substrate, and at the time indicated, octanoate was added. It is worth noting that the fluorescence decrease in the flavins upon the addition of octanoate (Fig. 1A) was almost identical in magnitude to that caused by anaerobiosis (not shown), while the fluorescence increase in the nicotinamide nucleotides (Fig. 1B) was only 60 % of that elicited by anaerobiosis. Infusion of KCl with resulting cessation of the contractions of the heart caused reduction of the flavoproteins (fluorescence decrease) and nicotin-

TABLE I

METABOLIC PARAMETERS OF FUNCTIONING AND KCl-ARRESTED PERFUSED HEARTS

The values are from freeze-clamped heart after 20-min open perfusion; means ±S.E.M. of 4–7 separate experiments.

	Functioning	Arrested
	(μmol/g dry weight)	
Citrate	0.465±0.062	1.331±0.265
Isocitrate	0.072±0.005	0.126±0.013
α-oxoglutarate	0.187±0.052	0.302±0.055
ATP	25.4 ±0.60	26.63 ±1.09
ADP	4.60 ±0.10	4.41 ±0.45
P_i	29.1 ±2.1	20.27 ±0.54
Creatine phosphate	42.12 ±2.1	49.82 ±0.54
	(μmol/g dry weight/min)	
Glucose uptake*	9.7	2.53
Glucose oxidation*	8.46	1.87
Oxygen consumption	26.8 ±2.5	11.0 ±2.2
$\frac{10^3 \cdot (\alpha\text{-oxoglutarate})\ (NH_3)}{3.87\ (\text{Glutarate})}$	1.58±0.73	1.18±0.33

* Separate experiments with recirculating perfusion, sampling of perfusate at time zero and 30 min.

amide nucleotides (fluorescence increase). These experiments also demonstrate the versatility of the optical geometry used in the fluorometry.

Since it is not possible to calibrate the fluorescence measurements on a percentage reduction scale, an attempt was made to measure the mitochondrial NAD/NADH ratio by using the glutamate dehydrogenase reaction as an indicator. When glucose was used as an external substrate, the redox state of the mitochondrial NAD system was found to be −314 mV and −318 mV in the beating and arrested heart respectively, so that the absolute value for the fluorometrically measurable redox change was quite small. However, glutamate dehydrogenase has quite a low activity in rat heart mitochondria [22], and is therefore not a very good redox indicator. This possibility means that the redox shift calculated is only a minimum value.

Fortunately, in spectroscopic measurements of the mitochondrial electron carriers above the rotenone-sensitive site, both the fully oxidized and fully reduced states can be recorded, which allows calibration of the redox measurements. If it is assumed that in the rotenone-inhibited state the cytochromes *c* and *a* are fully oxidized, and in the NaCN-inhibited state they are fully reduced, the steady state oxidation-reduction state of the cytochromes can be calculated. Experiments similar to those presented in Figs 2 and 3 showed that the oxidation-reduction potential of cytochrome *c* in the beating heart was +294 mV and that of cytochrome *a* +278 mV. When the heart was arrested, the redox potentials of cytochromes *c* and *a* rose to +315 mV and +316 mV respectively. In the calculations, the midpoint potential of cytochrome *c* was taken as $E_{m\ 7.0} = +235$ mV and that of cytochromes *a* as $E_{m\ 7.0} = +245$ mV [23]. Thus, the results show that in the perfused heart the cytochromes *c* and *a* are in near-equilibrium, which is actually to be expected on the basis of the

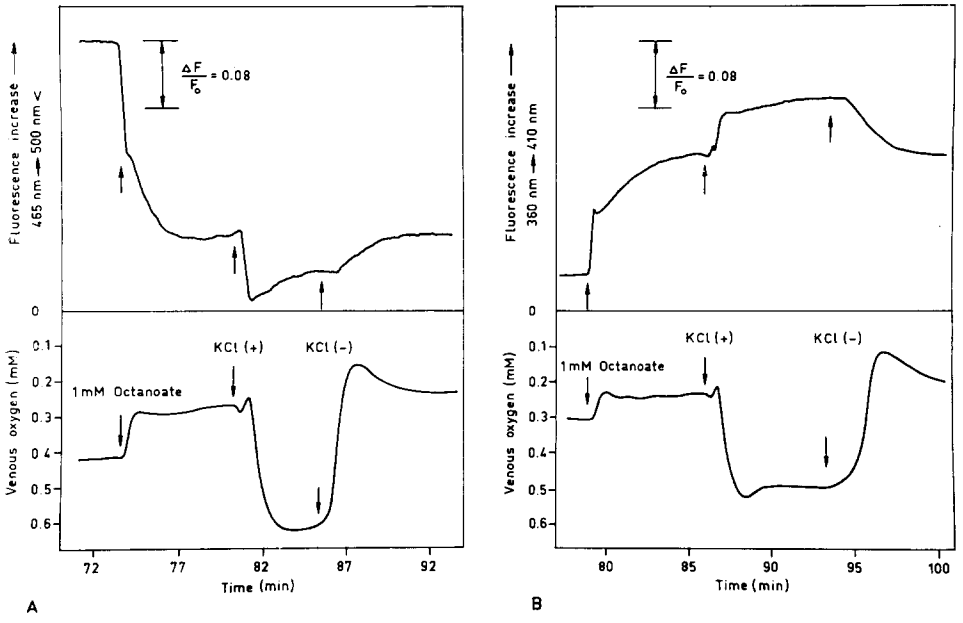

Fig. 1. Redox changes of the fluorescent flavoproteins and nicotinamide nucleotides in perfused rat heart upon substrate addition and cardiac arrest. Surface fluorescence of the isolated perfused heart (upper panel) and oxygen concentration in the venous perfusion fluid (lower panel) were monitored as described in the Experimental section. The signs KCl (+) and KCl (−) indicate the beginning and end of infusion of Krebs-Ringer bicarbonate solution containing 15 mM KCl. (A) flavoproteins; (B) nicotinamidenucleotides.

localization of the electron transport control sites in the cytochrome chain. The redox potential span between NAD/NADH and cytochrome *c* thus increased by 23 mV when the heart was arrested.

It has been shown previously that in suspensions of isolated hepatocytes there exists a near-equilibrium between the oxidation-reduction reactions in the respiratory chain and the phosphorylation state of the adenylate system [24]. Therefore we used parallel experiments to measure the concentrations of the adenosine nucleotides, inorganic phosphate and creatine phosphate in the perfused heart. Interesting findings were a decrease in the concentration of inorganic phosphate, quite small changes in the concentrations of the adenosine nucleotides and an increase in the concentration of creatine phosphate. The free energy change (ΔG) of ATP hydrolysis under the experimental conditions can be calculated from the equation

$$\Delta G = \Delta G'_0 + 2.303\, RT \log \frac{(\mathrm{ADP})(\mathrm{P_i})}{(\mathrm{ATP})} \qquad (1)$$

in which $\Delta G'_0$ is the standard free energy change of ATP hydrolysis (31.9 kJ/mol from ref. 25), R is the gas constant, and T is the temperature in K. If the phosphorylation state of the adenine nucleotides is in equilibrium with the redox state of the

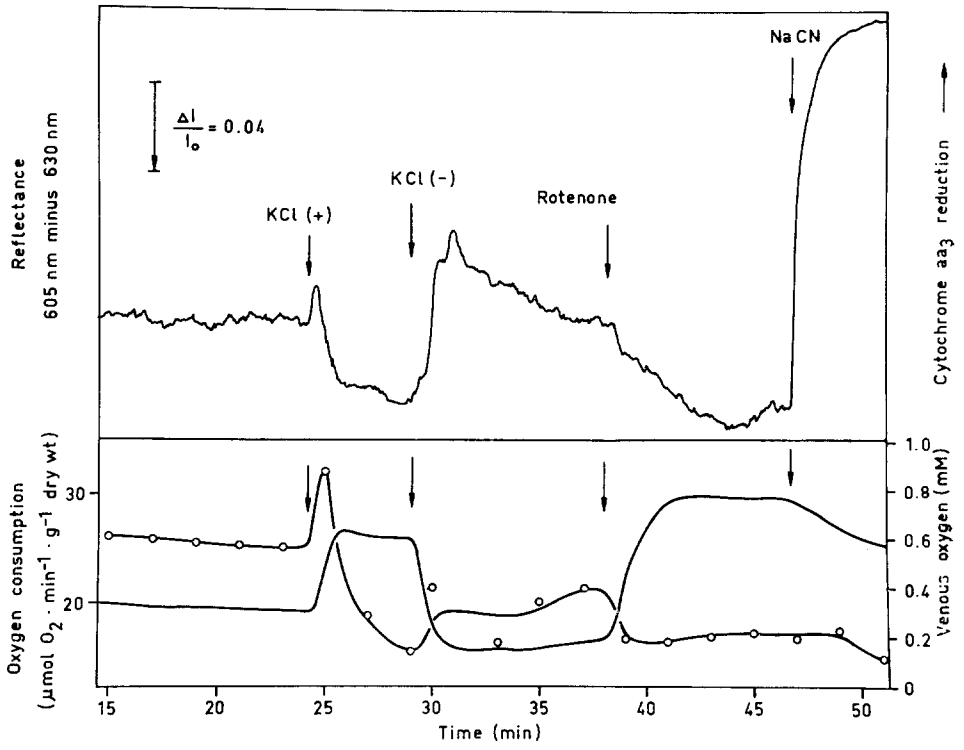

Fig. 2. Effect of cardiac arrest, rotenone and cyanide on the redox state of cytochrome *a* in an isolated perfused rat heart. Reflectance difference at the wavelengths 605 nm and 630 nm (upper panel) was monitored as described in the Experimental section. The heart was initially perfused with Krebs-Ringer bicarbonate (containing 10 mM glucose). The signs 'KCl (+)' and 'KCl (−)' indicate the beginning and end of perfusion with the same solution but containing 15 mM KCl. Rotenone, as a 5 mM ethanolic solution, was added to the perfusion fluid to give 10 μM final concentration, NaCN concentration was 2 mM. (Lower panel) ○–○, oxygen consumption; —, venous oxygen.

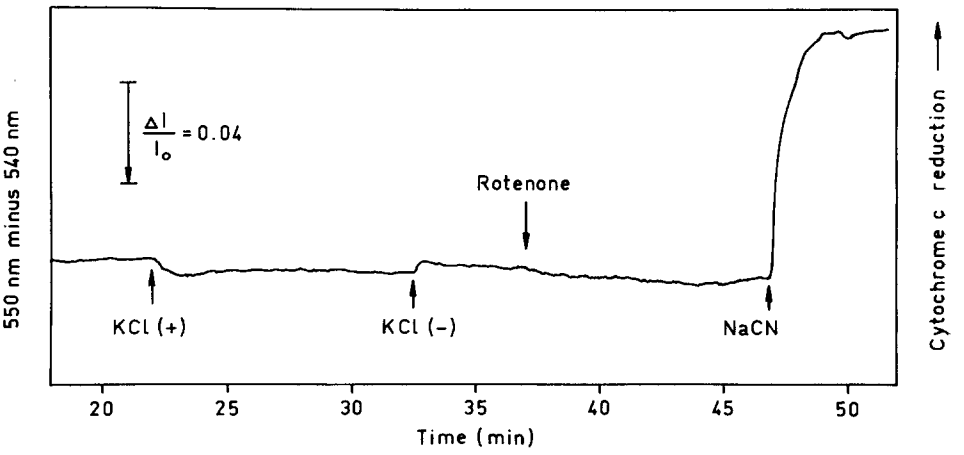

Fig. 3. Effects of cardiac arrest, rotenone and cyanide on the redox state of cytochrome *c* in an isolated perfused rat heart. Experimental conditions and symbols as in Fig. 2.

respiratory carriers, the free energy change of the oxidation-reduction reaction should be equal to that of the adenylate system. The free energy change of the former is

$$\Delta G = -nF\,\Delta E, \tag{2}$$

where n is the number of electrons transferred per molecule, F is the Faraday constant and ΔE is the potential difference between the donor and acceptor redox couples.

The ratio ATP/(ADP$\times$P$_i$) increased from $1.49 \cdot 10^3$ M^{-1} in the beating heart to $2.35 \cdot 10^3$ M^{-1}, calculated from total concentrations in the tissue when the heart was arrested by potassium excess (Table I). From Eqn 1 the free energy change of ATP hydrolysis under conditions prevailing in a beating heart is -101.5 kJ/2 mol, and from Eqn 2 the free energy change in the oxidation-reduction reactions between the span NAD/NADH and cytochrome *c* is -115.8 kJ/mol. However, it is known that muscle tissue contains an unusual concentration of binding sites for ADP, estimated at 0.6 mmol/kg [26]. When the "free" ATP/(ADP$\times$P$_i$) is calculated by making this correction, the phosphorylation potential rises from $9.68 \cdot 10^3$ M^{-1} to $21.88 \cdot 10^3$ M^{-1} on KCl-induced cardiac arrest. From these values the ΔG is 111.1 kJ/2 mol and 115.4 kJ/2 mol in the beating and arrested heart, respectively. This change in ΔG is equivalent to 21.2 mV across two phosphorylating sites, which compares favourably with the 23 mV change observed in the ΔE between NAD/NADH and cytochrome *c*. Therefore one can state that within the limits of experimental error there is a near-equilibrium between the electron carriers and the adenylate system in the perfused heart.

From Table I it can be seen that the depletion of P$_i$ is equal to the increase in creatine phosphate, which is in accordance with the calculated equilibria of creatine kinase and adenylate kinase reactions in muscle tissue [26]. The phosphorylation potential calculated from the whole tissue concentrations of the reactants is lower than that to be expected on the basis of a true equilibrium with the electron carriers. However, the concentrations of these reactants in the mitochondria in situ are not known.

It could be that the apparent redox changes in the cytochromes are due to a certain degree of tissue anoxia in the hemoglobin-free, perfused heart, resulting in large changes in the oxygen concentration when oxygen consumption alters drastically, as upon the arrest of the heart. The experiment depicted in Fig. 4 was conducted to check this possibility. At the moment marked "N_2+O_2" the perfusion fluid equilibrated with 95 % O_2+5 % CO_2 was changed to fluid equilibrated with 47.5 % O_2+47.5 % N_2+5 % CO_2. A decrease in the arterial oxygen concentration to half of its original value had no effect on the redox state of cytochrome *a*, indicating that conditions of liminal oxygen supply do not occur. At the moment marked "N_2" the gas bubbled into the perfusion fluid was changed to 95 % N_2+5 % CO_2. As the oxygen concentration approached zero, cytochrome *a* was slowly reduced, while on reoxygenation ("O_2") it was rapidly reoxidized. Therefore, one can assume that under the experimental conditions used in the previous experiments the oxygen supply was sufficient. Possibly due to diffusion of oxygen through the short lengths of plastic tubing in the perfusion apparatus, complete reduction of cytochrome *a* was not achieved under the nitrogen atmosphere, as is shown in the NaCN experiment in Fig. 4. As described under Experimental, the area of the heart surface used for the photometric measurements was not exposed to atmospheric air.

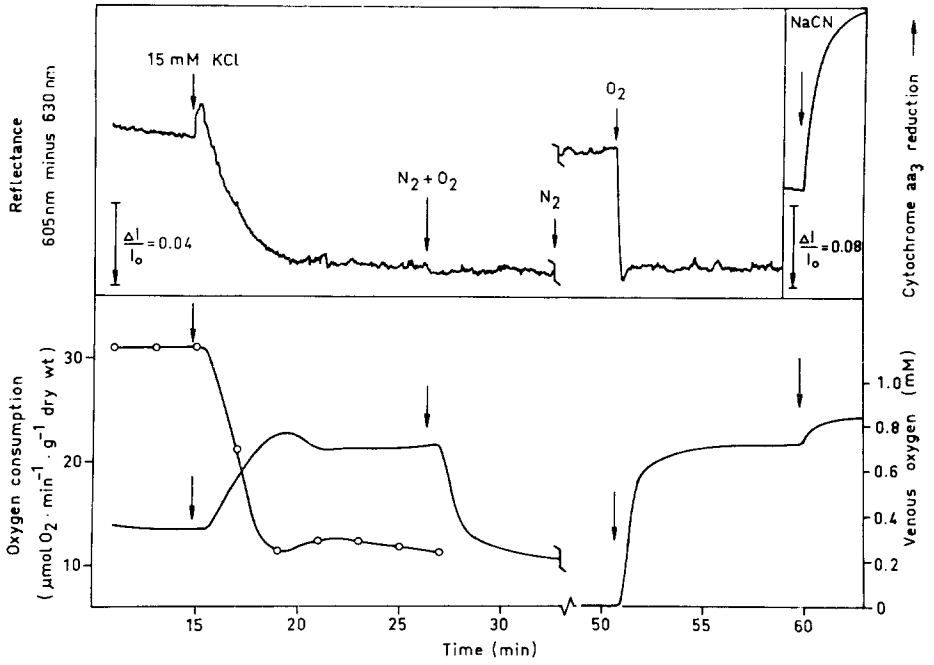

Fig. 4. Effects of arterial oxygen concentration on the redox state of cytochrome *a* in an isolated perfused rat heart. At time denoted "N_2+O_2" the perfusion fluid (Krebs-Ringer bicarbonate plus 10 mM glucose) in equilibrium with 95 % O_2+5 % CO_2 was changed to fluid in equilibrium with 47.5 % O_2+47.5 % N_2+5 % CO_2, at time 'N_2' the gas phase was changed to 95 % N_2+5 % CO_2, and at time 'O_2' the perfusion fluid was changed to one in equilibrium with 95 % O_2+5 % CO_2. Other symbols as in Fig. 2.

The oxidation of cytochromes *a* and *c* upon diminution of oxygen consumption is also compatible with the concept of regulation of cellular respiration by means of the terminal irreversible reaction between cytochrome oxidase and oxygen. However, the contribution of cytochrome a_3 to the absorbance change at 605 nm under these experimental conditions is not known. Probably cytochrome a_3 is largely oxidized [27]. If it is assumed that the third phosphorylation site is between cytochromes *a* and a_3 and that the equilibrium between the adenylates and electron carriers also holds for this site, the direction of the redox changes of cytochromes *a* and a_3 should be the same in the perfused heart. This is actually in accordance with the experiments shown in Figs 5 and 6. The oxygen consumption of the arrested heart increased considerably upon the infusion of the uncoupler, carbonyl cyanide *m*-chlorophenylhydrazone (5 μM) into the perfusion medium, and simultaneously the cytochrome *a* was reduced. In fact, when the percentage reduction is plotted against oxygen consumption, a straight line is obtained (Fig. 6). It is obvious that this experimental correlation does not mean a causal relationship. Unfortunately, the oxidation-reduction state of cytochrome a_3, one of the reactants of the irreversible step of oxygen reduction, cannot be directly measured with the instrumentation available at present. That the change in the flux is related to a change in the redox state is demonstrated in Fig. 7, which shows that a prior addition of rotenone

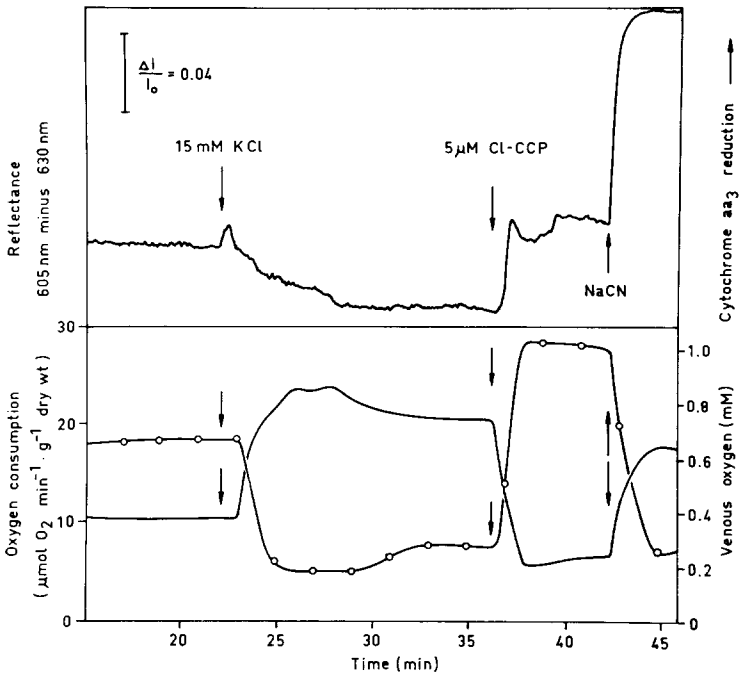

Fig. 5. Effect of an uncoupler on the redox state of cytochrome *a* in an isolated perfused rat heart. Carbonyl cyanide *m*-chlorophenylhydrazone (Cl-CCP), as a 2 mM ethanolic solution, was added to the perfusate to give a final concentration of 5 μM. NaCN concentration was 2 mM. Other conditions and symbols as in Fig. 2.

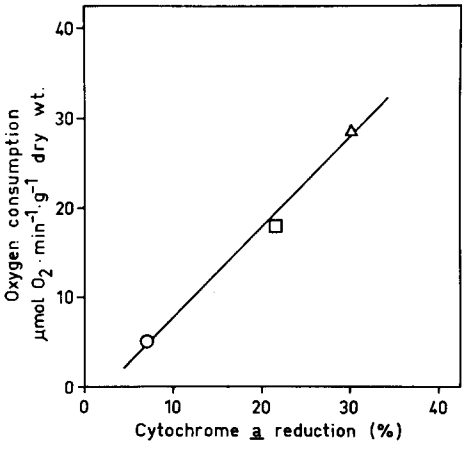

Fig. 6. Correlation between the oxygen consumption and reduction of cytochrome *a* in perfused rat heart. Experimental conditions as in Fig. 5. (○) KCl-arrested heart, (□) beating heart, (△) heart in the presence of 5μM carbonyl cyanide m-chlorophenylhyd azone. Percentage reduction of cytochrome *a* was estimated on the basis of rotenone and cyanide inhibition data as explained under Experimental.

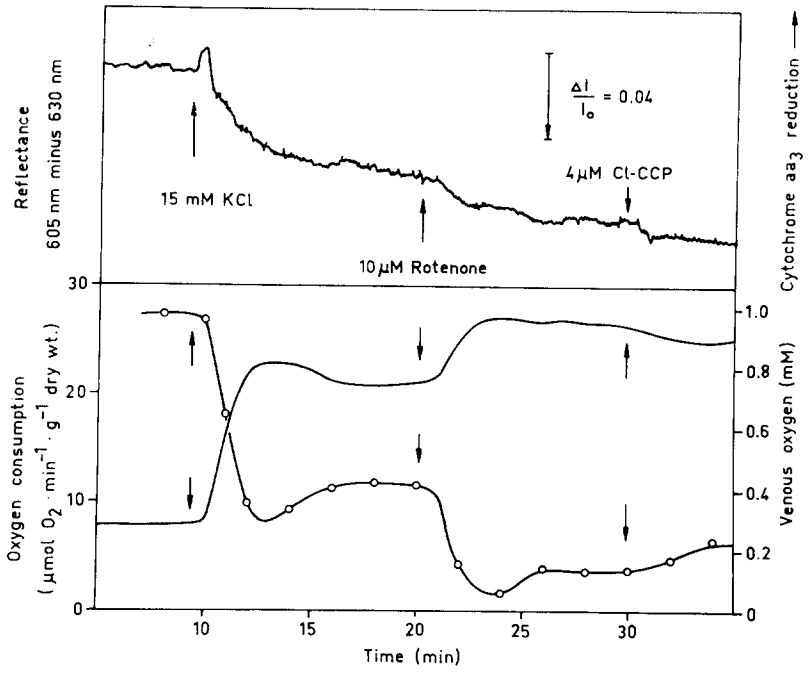

Fig. 7. Effect of an uncoupler on the redox state of cytochrome *a* in a rotenone-inhibited arrested heart. Rotenone as a 5 mM ethanolic solution was added to the perfusion fluid to give 10 μM final concentration. Other conditions and symbols as in Fig. 5.

to the perfusion fluid abolishes the effects of the uncoupler on the redox state of cytochrome *a*.

10 mM glucose was the only external oxidizable substrate for the heart in the experiments shown in Figs 2 to 7. From Table I it may be calculated, that under these conditions the oxygen consumption can mostly be accounted for by glucose oxidation in the beating heart, while in the arrested heart 51 % of the oxygen consumption can similary be accounted for by glucose oxidation. This means that under conditions of respiratory inhibition the glycolysis in inhibited very effectively. Glucose oxidation is inhibited more than the glycolytic flux, but the latter, too, shows very effective regulation.

The present results have some important implications for the regulatory mechanisms in the energy metabolism at two levels:

(a) Cellular respiration in intact tissue is apparently proportional to the reduction grade of electron carriers proximal to the site of oxygen reduction. The redox state of the carriers is a function of the energy state of the adenylate system. These observations may be regarded as experimental evidence of the applicability of the thermodynamic equilibrium hypothesis of Klingenberg [28], more recently confirmed by Wilson et al. [24, 29] to intact myocardium.

Muscle tissue is perhaps the only tissue in which "physiological" inhibition of cellular respiration occurs in sufficient magnitude to be used as a tool in studies on the respiratory control of intact tissue.

(b) Under conditions of glucose oxidation by the perfused heart an effective feed-back inhibition of the glycolytic and tricarboxylic acid pathways is functioning. The diminishing flux in the respiratory chain causes accumulation of citric acid cycle intermediates. However, no accumulation of pyruvate and only a small accumulation of acetyl-CoA occurs [30] (Hiltunen and Hassinen, in preparation). The uptake of glucose in a recirculating perfusion was inhibited to a greater extent than that of oxygen. It was found that great losses of lactate and pyruvate occurred in an open perfusion, compared with the accumulation of lactate and pyruvate in a recirculating system. Calculations on the basis of the results obtained with the latter showed that almost all the oxygen consumption of the beating heart could be accounted for by glucose oxidation, but this result must be interpreted with caution. The difference between the glucose concentrations of the arterial and venous perfusate was too small to be determined with accuracy in open perfusion.

The results also show that the changes in the concentration of the adenine nucleotides, known modulators of some enzymes of the tricarboxylic acid cycle are probably not sufficient in heart tissue to significantly affect the activities of the individual enzymes of the tricarboxylic acid cycle [31].

Citrate synthase is known to be particularly sensitive to the adenylate. However, unequivocal evidence for the significance of such inhibition in living cells is lacking (for the complexities of the control of citrate synthase see ref. 32). The present results could be better reconciled with an inhibition of the cycle at the oxidationreduction reactions, not at the citrate synthase reaction, that associated with substrate entry to the cycle. Accumulation of intermediates before the oxoglutarate dehydrogenase step suggest that effective control also exists at this site under these experimental conditions. However, it must be borne in mind that an accumulation of citric acid cycle intermediates in cardiac tissue is possible from other carbon sources e.g. amino acids [33], so that the localization of control sites is not a very straightforward matter. On the other hand, the changes in the "phosphorylation potential" are sufficient to affect the redox state of the respiratory carriers by means of equilibrium reactions. The other phenomena of regulation in the tricarboxylic acid cycle occur secondary to the changes in the redox state of the carriers, probably those of the NAD couple, or are mediated by other high-energy compounds than ATP [34]. Substrate entry into the tricarboxylic acid cycle does not seem to be a significant regulator of the terminal oxidations under the experimental conditions used in the present study.

ACKNOWLEDGEMENTS

This investigation was supported by grants from The Sigrid Juselius Foundation, Finland and The Medical Research Council of the Academy of Finland. The authors are indebted to Mrs. Maija-Liisa Takalo for skillful technical assistance.

REFERENCES

1 Chance, B. and Williams, G. R. (1955) J. Biol. Chem. 217, 383–394
2 Lardy, H. A. and Wellman, H. J. (1952) J. Biol. Chem. 195, 215–224
3 Scholz, R., Thurman, R. G., Williamson, J. R., Chance, B. and Büche., T. (1970) J. Biol. Chem. 245, 3263–3268

4 Bing, R. J. (1965) Physiol. Rev. 45, 171–213
5 Krebs, H. A. and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33–36
6 Langendorff, O. (1895) Pflügers Arch. Gesamte Physiol. 61, 291–332
7 Hassinen, I. E. Härkönen, M. H. A. and Ylikahri, R. H. (1974) Brain Res. 70, 301–312
8 Chance, B. (1951) Rev. Sci. Instr. 22, 634–638
9 Wollenberger, A., Ristau, O. and Schoffa, G. (1960) Pflüger's Arch. Gesamte Physiol. 270, 399–412
10 Williamson, J. R. and Corkey, B. (1969) in Methods in Enzymology (Colowick, S .P. and Kaplan, N. O., eds), Vol. 13, pp. 439–513, Academic Press, New York
11 Adam, H. (1963) in Methods of Enzymatic Analysis (Bergmeyer, H. V., ed.), pp. 573–577, Academic Press, New York
12 Lamprecht, W. and Trautschold, I. (1970) in Methoden der Enzymatischen Analyse (Bergmeyer, H. V., ed.), pp. 2024–2033, Verlag Chemie, Weinheim
13 Gruber, W. and Moellering, H. (1966) Biochem. Z. 346, 78–88
14 Goldbe g, N. D. and Passonneau, J. V. (1970) in Methoden de Enzymatischen Analyse, pp. 1532–1535, Verlag Chemie, Weinheim
15 Narins, R. G. and Passonneau, J. V. (1970) in Methoden der Enzymatischen Analyse (Bergmeyer, H. V., ed.), pp. 1540–1543, Verlag Chemie, Weinheim
16 Gawehn, K. (1970) in Methoden der Enzymatischen Analyse (Bergmeyer, H. V., ed.), pp. 2156–2160, Verlag Chemie, Weinheim
17 Bernt, E. and Bergmeyer, H. V. (1970) in methoden der Enzymatischen Analyse (Bergmeyer, H.V., ed.) pp. 1659–1663, Verslag Chemie, Weinhein
18 Kun, E. and Kea ney, E. B. (1970) in Methoden dei Enzymatischen Analyse (Bergmeyer, H. V., ed.), pp. 1748–1752, Verlag Chemie, Weinheim
19 Hohorst, H.-J. (1963) in Methods in Enzymatic Analysis (Bergmeyer, H. V., ed.), pp. 266–270, Academic Press, New York
20 Bücher, T., Czoh, R., Lampsecht, W. and Latzko, E. (1963) in Methods in Enzymatic Analysis, (Bergmeyer, H. V., ed.), pp. 253–259, Academic Press, New York
21 Hugget, A. St. G. and Nixon, D. A. (1957) Biochem. J. 66, 12P
22 Pette, P., Klingenberg, M. and Bücher, Th. Biochem. Biophys. Res. Commun. (1962) 7, 425–429
23 Dutton, P. L., Wilson, D. F. and Lee, C.-P. (1970) Biochemistry 9, 5077–5082
24 Wilson, D. F., Stubbs, M., Veech, R. L., Erecinska, M. and Krebs, H. A. (1974) Biochem. J. 140, 57–64
25 Guyun, R. and Veech, R. L. (1973) J. Biol. Chem. 248, 6966–6972
26 McGilvery, R. W. and Murray, T. W. (1974) J. Biol. Chem. 249, 5845–5850
27 Vanneste, W. H., Ysebaert-Vanneste, M. and Mason, H. S. (1974) J. Biol. Chem. 249, 7390–7401
28 Klingenberg, M. (1961) Biochemistry 2, 335, 243–262
29 Owen, C. S. and Wilson, D. F. (1974) Arch. Biochem. Biophys. 161, 581–591
30 Fath, P. H. and Kako, K. J. (1973) J. Mol. Cell. Cardiol. 5, 329–373
31 Atkinson, D. C. (1968) in The Metabolic Role of Citrate (Goodwin, R. W., ed.), pp. 23–39, Academic Press, London
32 Srere, P. A. (1971) Adv. Enzyme Regul. 9, 221–233
33 Davis, E. J. and Bremer, J. (1973) Eur. J. Biochem. 38, 86–97
34 LaNoue, K. F., Walajtys, E. I. and Williamson, J. R. (1973) J. Biol. Chem. 248, 7171–7183