SUBCELLULAR DISTRIBUTION OF PHOSPHAGENS IN ISOLATED PERFUSED RAT HEART

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1. Introduction

It has been suggested that the oxygen consumption of the mitochondrial respiratory chain is regulated by the extramitochondrial ATP/ADP × P_i ratio and that a near-equilibrium exists between the oxidation–reduction reactions of the mitochondrial respiratory chain and the phosphorylation of cytosolic ADP [1]. Data on the situation in intact tissues have been largely obtained by measurements of total average concentrations of the reactants [2], but corroboration of the near-equilibrium hypothesis [3] of respiratory control needs data on the concentrations of the reactants in specified intracellular compartments.

The isolated perfused rat heart has been employed in studies on the regulation of cellular respiration, and total average concentrations of ATP, ADP, P_i, creatine and creatine phosphate used in calculations of the extramitochondrial phosphorylation potential [4,5]. Here, advantage has been taken of the method of tissue fractionation in non-aqueous media [6] to measure the cytosolic and mitochondrial phosphorylation potentials. The results show that the error in estimating the cytosolic phosphorylation potential from total tissue concentration of the reactants is quite small when calculated from the creatine kinase equilibrium. The results are compatible with a near-equilibrium between the mitochondrial respiratory chain and the cytosolic adenylate system. Changes in the distribution of the adenylates across the mitochondrial membrane upon transitions in the cellular ATP consumption indicate that the mitochondrial membrane potential and transmembrane pH difference also change.

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2. Material and methods

2.1. *Animals and perfusion method*

Female Sprague-Dawley rats from the Department's own stocks were used. The rats (200–250 g) were anesthetized with intraperitoneal pentobarbital and injected intravenously with 500 IU of heparin before excision of the heart. Isolated hearts were perfused by the Langendorff procedure [7] with Krebs-Ringer bicarbonate solution [8] equilibrated with O_2/CO_2 (19:1) and containing 2.5 mM Ca^{2+}. Perfusion time was 30 min and the hydrostatic pressure 7.85 kPa (80 cm H_2O). When studying arrested hearts, they were first perfused for 15 min in the usual manner, after which $[K^+]$ was increased to 16 mM (the $[Na^+]$ being reduced accordingly) and the perfusion was continued for another 15 min.

2.2. *Tissue sampling and fractionation*

The heart was quick-frozen with aliminium clamps precooled with liquid nitrogen and freeze-dried at −55°C. Metabolite distribution in the tissue was determined principally according to [6]. The dry tissue was homogenized in heptane/carbon tetrachloride mixture in a Bühler homogenizer thermostated at −36°C. The total homogenizing time was 3 h in 90 s periods separated by 90 s intervals for cooling. The homogenate was filtered through a column of glass beads and layered on top of a discontinuous gradient of carbon tetrachloride in heptane, extending from a specific gravity of 1.28–1.33 in 4 layers. The gradients, in nitrocellulose tubes, were centrifuged at 13 000 rev./min in a Spinco SW-41 rotor for 2 h. The fractions collected were divided into two portions, evaporated to dryness and analyzed for protein, citrate synthase,

phosphoglycerate kinase and the metabolites under study.

2.3. *Analytical methods*

Citrate synthase was determined by the method in [9] and phosphoglycerate kinase according to [11] and creatine phosphate in the same assay by the subsequent addition of ADP and creatine kinase. ADP was assayed according to [12]. Conventional enzymatic methods of creatine determination gave values which were only 1/3rd of those obtained in direct acid extracts of the tissue. The decreased creatine concentrations were found to be due to creatinine formation during the tissue fractionation procedure. When creatininase (creatinine aminohydrolase) was included in the assay medium, creatine concentrations measured were comparable to those in acid extracts of freeze-clamped isolated perfused hearts. Creatine was measured in the same cuvette as ADP by the subsequent addition of creatininase and creatine kinase. P_i was assayed as in [13]. Protein was determined according to [14].

2.4. *Determination of intracellular and mitochondrial water*

Intracellular water was calculated by first measuring the extracellular water by perfusing the heart in a closed system for 0.5–2 h with a medium containing inulin–[^{14}C]carboxylic acid, which was subtracted from the total tissue water calculated from the wet and dry weights of the tissue. Mitochondrial matrix water was measured in separate experiments with suspensions of rat heart mitochondria prepared according to [15]. They were incubated in the presence of ^3H_2O and inulin–[^{14}C]carboxylic acid, and the matrix water was taken as the ^{3}H-accessible minus ^{14}C-accessible space, and was found to be 1.80 ± 0.27 μl/mg mitochondrial protein. The mitochondrial protein content of the tissue was determined principally by the same technique as the metabolite distribution. The cytosolic water space was taken as the intracellular minus mitochondrial water, and the cytosolic marker enzyme and cytosolic metabolites were taken to be evenly distributed in the extramitochondrial space.

3. Results and discussion

3.1. *Compartmentation*

The compartmentation of ATP, ADP, P_i, creatine phosphate (CrP) and creatine (Cr) are presented in table 1. The total cytosolic ATP concentration was 1.4-fold in the beating heart and 2.5-fold in the arrested heart as compared with the intramitochondrial concentration. Of the total amount of ATP, 85% was in the cytosol in the beating heart and 91% in the arrested heart.

Table 1
Subcellular distribution of adenylates, creatine, creatine phosphate and inorganic phosphate in beating and potassium-arrested isolated perfused rat hearts

	Concentration (mM)	
	Beating heart	Arrested heart
ATP		
cytosolic	8.08 ± 1.00 (6)	7.16 ± 0.56 (10)
mitochondrial	5.96 ± 0.17 (6)	2.82 ± 0.56 (10)
ADP		
cytosolic	1.46 ± 0.32 (5)	1.44 ± 0.30 (6)
mitochondrial	2.20 ± 0.59 (5)	2.70 ± 0.49 (6)
Creatine phosphate		
cytosolic	14.00 ± 2.12 (3)	18.99 ± 3.04 (5)
mitochondrial	7.20 ± 1.66 (3)	3.74 ± 1.18 (5)
Creatine		
cytosolic	15.63 ± 2.15 (2)	11.28 ± 0.28 (2)
mitochondrial	6.10 ± 0.90 (2)	7.57 ± 1.66 (2)
Inorganic phosphate		
cytosolic	3.73 ± 1.08 (6)	2.55 ± 0.42 (5)
mitochondrial	4.82 ± 0.87 (6)	6.77 ± 1.34 (5)

The results are means ± SEM for no. expt. in parenthesis

Expressed as the total concentration, the mitochondrial ADP concentration was 1.5-fold in the beating heart and 1.9-fold in the arrested heart as compared with the cytosolic concentration. As seen below, the concentration gradient of free ADP across the mitochondrial membrane was steeper. CrP and Cr were located mainly in the cytosol, and P_i mainly in the mitochondria.

3.2. *Free energy relations of ATP hydrolysis in the cytosol and mitochondria*

It is known that in muscle tissue most of the cytosolic ADP is tightly bound to the contractile proteins [16], so that free ADP represents only a fraction of the total. It is probable that binding of CrP, Cr and P_i to cytosolic structures is low. When the equilibrium

constant of Mg^{2+}-activated creatine kinase is taken as $2.81 \times 10^{10}/(H^+)$ [17] and the intracellular pH as 7.4, according to the ^{31}P NMR data [18], the equilibrium constant K_{ck} of creatine kinase becomes 7.058×10^{-3}. The free energy change of cytosolic ATP hydrolysis can be calculated from eq. (1):

$$\Delta G_{ATP} = \Delta G'_0 + RT \cdot \ln \frac{[CrP_c]}{[Cr_c] \cdot [P_{i\,c}]} \cdot \frac{1}{K_{ck}} \qquad (1)$$

where $\Delta G'_0 = -31.9$ kJ/mol under conditions prevailing intracellularly [19], and $[CrP_c]$, $[Cr_c]$ and $[P_{i\,c}]$ are the cytosolic concentrations of creatine phosphate, creatine and P_i, respectively, and K_{ck} is the equilibrium constant of creatine kinase. ΔG_{ATP} is therefore −58.8 kJ/mol in the beating heart and −61.4 kJ/mol in the potassium-arrested heart. Only free ADP concentrations in the μM range are obtained from the creatine kinase equilibrium for the myocardial cytosol (table 2).

Under conditions identical to those used here, the ΔG in the oxidation–reduction reaction between NADH and cytochrome *c* has been found to be −115.8 kJ/2 e^- and −122.2 kJ/2 e^- in the beating and potassium-arrested heart, respectively [4]. The observed ΔG value of cytosolic ATP hydrolysis here was −117.6 kJ/2 mol (for the ATP/2 e^- = 2 between NADH and cytochrome *c*) and −122.8 kJ/2 mol in the beating and arrested, heart respectively; i.e., within experimental error, there is an equilibrium between the redox reactions in the mitochondrial respiratory chain and ATP synthesis.

Table 2
Mitochondrial–cytosolic concentration gradients, and cytosolic phosphorylation state of the adenylates in beating and potassium-arrested isolated perfused rat hearts

	Beating heart	Arrested heart
	(Concentration ratio)	
ATP cytosolic/mitochondrial	1.36	2.54
ADP cytosolic/mitochondrial	0.66	0.53
Creatine phosphate cytosolic/mitochondrial	1.93	5.08
Creatine cytosolic/mitochondrial	2.56	1.49
Inorganic phosphate cytosolic/mitochondrial	0.77	0.38
	(Concentration, mM)	
Free ADP[a] cytosolic	0.064	0.030
	(Molar ratio)	
[ATP]/[ADP] mitochondrial	2.71	1.04
[ATP]/[free ADP] cytosolic	288.6	550.8
	(kJ/mol)	
ΔG_{ATP} cytosolic	−58.8	−61.4

[a] Calculated from the creatine kinase equilibrium

3.3. *Energy-linked changes in the distribution of adenylates and phosphate across the mitochondrial membrane*

The ATP/ADP ratio is much lower in the mitochondria than in the cytosol (table 2), and in accord with measurements made on a suspension of isolated mitochondria [20]. The ATP/ADP exchange in the inner mitochondrial membrane is largely electrogenic [21], so that the membrane potential (negative inside) across the mitochondrial membrane may be the driving force of the asymmetry of the adenylate distribution. Upon diminution of the cellular ATP consumption during cardiac arrest a decrease in mitochondrial ATP and an increase in mitochondrial ATP and an increase in mitochondrial ADP occured. This may indicate that an increase in the mitochondrial membrane potential occurs during an increase in the energy state of the cell. The increase in the mitochondrial ADP concentration during a decrease in the cytosolic ADP concentration following a decrease in the mitochondrial respiratory rate does not support the view that the oxygen consumption is regulated by the adenylate carrier [20,22].

If phosphate is distributed across the mitochondrial membrane according to the pH gradient [23], the latter is 0.06 pH units (alkaline inside) in the beating heart and 0.23 pH units (alkaline inside) in the arrested heart.

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