^{31}P-NMR studies of respiratory regulation in the intact myocardium

Arthur H.L. From°*, Marc A. Petein°, Steven P. Michurski+, Stevan D. Zimmer° and Kâmil Uğurbil+*

+*Departments of Biochemistry and the Gray Freshwater Biological Institute, University of Minnesota, Navarre, MN 55392* and °*Cardiovascular Division, Department of Medicine, Minneapolis VA Medical Center and University of Minnesota, 54th St. and 48th Ave. S., Minneapolis, MN 55417, USA*

Received 10 June 1986, revised version received 11 July 1986

The mechanism by which mitochondrial respiration is coupled to ATP consumption in intact tissues is unclear. We determined the relationship between high-energy phosphate levels and oxygen consumption rate in rat hearts operating over a range of workloads and perfused with different substrates. With pyruvate + glucose perfusion, ADP levels were in general very low, and varied with MVO_2 yielding an apparent K_m of 25 ± 5 μM, suggesting regulation of oxidative phosphorylation through availability of ADP. In contrast, with glucose perfusion in the presence or absence of insulin, ADP levels, ADP/ATP ratio or the phosphate potential were relatively constant over the workload range examined and generally not correlated with alterations in MVO_2; it is suggested that under these conditions, carbon substrate delivery to the mitochondria may control mitochondrial respiration. The common feature of both of the suggested regulatory mechanisms is substrate limitation which, however, is exercised at different metabolic points depending on the carbon substrate available to the myocardium.

^{31}P-NMR *Respiratory regulation* *Substrate dependence* *(Rat myocardium)*

1. INTRODUCTION

Alterations in the ATP demand of aerobic tissue are met primarily through concurrent changes in the rate of oxidative phosphorylation. In cardiac muscle, this interrelationship is manifested as the well recognized coupling between contractile activity and myocardial velocity of O_2 consumption (MVO_2) [1]. Current understanding of the mechanism responsible for this coupling remains controversial and is largely derived from studies on respiratory regulation of isolated mitochondria. Approx. 30 years ago, Chance and Williams [2] reported that the respiration rate of mitochondria was determined by the concentration of exogenous ADP and obtained an apparent K_m of 20–30 μM for ADP utilization. Subsequently, phosphorylation potential (PP) which is equal to [ATP]/[ADP][P_i], and ATP/ADP ratio were also proposed as the regulatory parameters in mitochondrial respiratory control [3–5].

The applicability of any of these three potential mechanisms in intact cells has not been rigorously tested previously; earlier studies were severely limited by the inability of extraction techniques to measure free cytosolic concentrations of the phosphorylated metabolites, in particular, of ADP [6]. Therefore, we undertook a ^{31}P-NMR study of perfused rat hearts subjected to different workloads. It has been recognized that the level of myocardial high-energy phosphates depends on the carbon substrate utilized by the heart [7,8]. Therefore, these studies were conducted using three different exogenous carbon substrate condi-

* To whom correspondence should be addressed

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

tions, namely, glucose (G), glucose + insulin (GI), and pyruvate + glucose (PG).

2. MATERIALS AND METHODS

In these studies, isovolumic, Langendorff-perfused hearts obtained from 400–500 g rats were used. The methods for perfusion, ventricular pressure measurement (via an intraventricular balloon), and MVO_2 determination have recently been detailed [5]. The myocardial ATP and CP content were obtained using fully relaxed ^{31}P-NMR spectra and a reference signal. ADP content was calculated from the creatine kinase equilibrium constant ($[ATP][Cr]/[ADP][H^+][CP] = 1.66 \times 10^9\ M^{-1}$) [9], using a cytosolic pH value of 7.05 [10] and a myocardial creatine (Cr) plus creatine phosphate (CP) content of 68.5 μmol/g dry wt [11]. Cytosolic concentrations were calculated using a cytosolic volume of 0.44 ml/g wet wt [12] and a g wet wt/g dry wt ratio of 5.7 ± 0.1 ($n = 10$), obtained under our experimental conditions. The product of systolic pressure and heart rate (RPP) was used as an index of myocardial mechanical output [1]. Each heart, irrespective of the carbon substrate used, was subjected to six consecutively increasing levels of workload and MVO_2 achieved by altering the heart rate (HR), end-diastolic pressure (EDP) and exposure to an inotropic agent (dobutamine; Dobutrex® , Eli Lilly, Indianapolis, IN). These were characterized by: (i) HR = 180, EDP = 8 mmHg; (ii) HR = 300, EDP = 8 mmHg; (iii) HR = 300, EDP = 8 mmHg plus 40 ng/ml dobutamine; (iv) HR = 450, EDP = 17–20 mmHg plus 40 ng/ml dobutamine; (v) HR = 450, EDP = 17–20 mmHg, 80 ng/ml of dobutamine and (vi) HR = 600, EDP = 17–20 mmHg, 80 ng/ml of dobutamine. The EDP was set by adjusting the intraventricular balloon volume. In these hearts, the right atrium was partially removed in order to reduce the spontaneous rhythm and permit pacing at low heart rates. Glucose concentration was 15 mM in G and GI perfusion. Insulin was 20 IU/l. In PG perfusion, both pyruvate and glucose were present at 10 mM.

3. RESULTS AND DISCUSSION

Fig.1 illustrates typical spectra obtained from hearts under the three substrate conditions at a fairly high RPP (~60000 mmHg/min). Bioenergetic and mechanical data obtained at the lowest and highest levels of mechanical output are given in table 1. Over the range of RPP attained, the relationship between MVO_2 and RPP was linear and comparable for the different substrate conditions as previously shown [1]. The relationship between MVO_2 and ADP concentrations is illustrated as a double-reciprocal plot in fig.2; in this representation, a straight line with a slope of K_m/V_{max} and intercept of $1/V_{max}$ would be expected if ADP availability is the rate-determining factor in respiration.

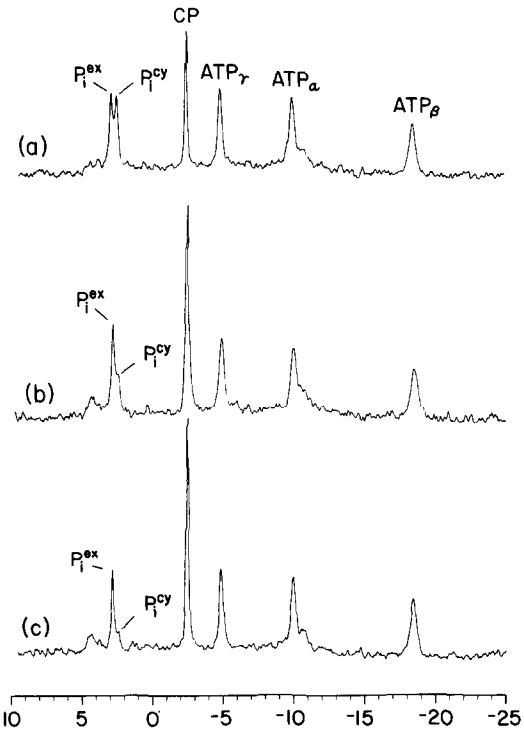

Fig.1. 146.1 MHz ^{31}P-NMR spectra of isovolumic, Langendorff-perfused rat hearts at a moderately high workload achieved with HR = 300, EDP = 8, and 40 ng/ml of dobutamine in the perfusate using G (a), GI (b), and PG (c) as the carbon substrate. The spectra are the sum of 40 free induction decays obtained with 90° pulses and 15 s repetition time.

It is evident from these data (table 1, fig.2) that for a given MVO_2, ATP contents were comparable, whereas cytosolic P_i and ADP levels, ATP/ADP ratio and the PP were significantly dif-

Table 1

RPP, MVO_2, ATP, ADP and P_i content, and PP at the lowest (i) and the highest (vi) workloads examined in isovolumic Langendorff-perfused rat hearts supplied with exogenous substrates G, GI and PG[a]

	G perfused (n = 8)		GI perfused (n = 6)		PG perfused (n = 8)[b]	
	i	vi	i	vi	i	vi
RPP (10^3 mmHg)	22 ± 1	91 ± 6	21 ± 2	100 ± 6	20.7 ± 0.8	91 ± 2
MVO_2 (μmol/min per g dry wt)	37 ± 2	83 ± 3	30 ± 2	84 ± 4	44 ± 3	84 ± 3
ATP (μmol/g dry wt)	25 ± 1	20 ± 1	23 ± 2	21 ± 1	27 ± 2	23 ± 1
ADP (nmol/g dry wt)	139 ± 17	130 ± 12	94 ± 12	91 ± 11	25 ± 9	69 ± 7
P_i (μmol/g dry wt)[c]	10 ± 2	24 ± 4	5 ± 1	18 ± 5	0.9 ± 0.5	10 ± 2
Phosphate potential (PP)[d] (10^3 M^{-1})	46 ± 9	16 ± 3	116 ± 25	33 ± 9	3010 ± 1147	82 ± 15

[a] All values are mean ± SE

[b] For the PG perfused hearts, n was 12 for the ATP and ADP data

[c] P_i content was measured at one workload in hearts perfused with P_i-free media under otherwise identical conditions (n = 5 for all substrates; P_i content at other workloads were obtained from these data, and the change in P_i content measured from difference spectra in hearts perfused under the normal experimental protocol

[d] Errors were calculated as SE from the errors in the ATP, ADP and P_i measurement

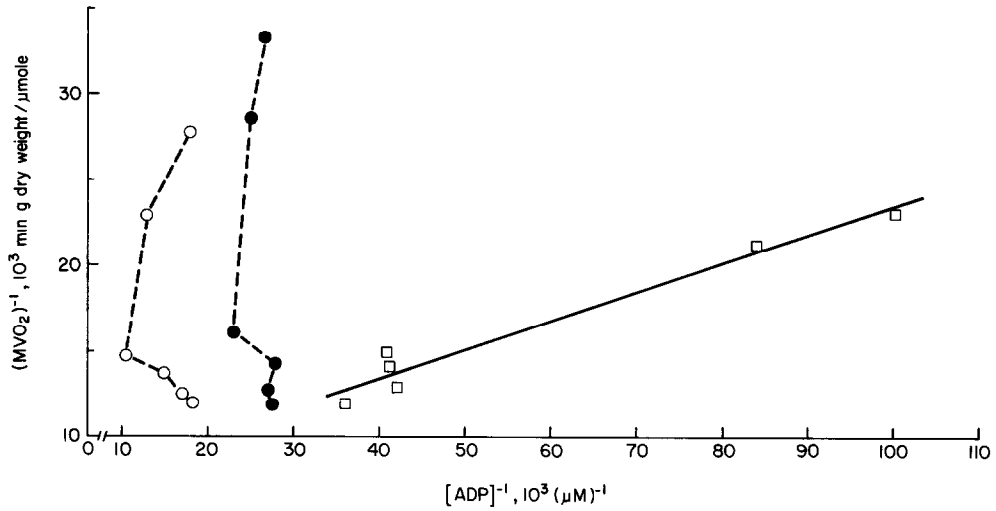

Fig.2. Double-reciprocal plot of $(MVO_2)^{-1}$ vs $[ADP]^{-1}$ in G (○), GI (●), and PG (□) perfused hearts. The solid line was obtained by a linear least-squares fit to the PG data.

ferent for the three different exogeneous substrate conditions. This implies that none of the three previously promoted entities (i.e. ADP content, ATP/ADP ratio and the PP) serve as a universally applicable parameter that regulates mitochondrial respiration in the intact myocardium. Lack of a general correlation between PP and mitochondrial respiration in intact perfused hearts has also been noted previously [8].

For PG substrate conditions, there was a linear relationship between $(MVO_2)^{-1}$ and $[ADP]^{-1}$ which gave an apparent K_m of 25 ± 5 μM and V_{max} of 148 ± 24 μmol O_2/min per g dry wt. This K_m is very similar to values obtained from isolated

mitochondrial studies where ADP availability was shown to be the rate-limiting factor in respiration [2,13].

Under PG substrate conditions, both the ATP/ADP ratio and the PP decreased as the workload was increased (table 1). These trends are similar to those documented in previous studies with isolated mitochondria; such correlations have been the basis for attributing to these two parameters a possible regulatory role in respiratory control. However, it was recently shown in isolated mitochondrial suspensions that the usual inverse relationship observed between respiration rate and the ATP/ADP ratio, or the PP could be reversed by altering the means of ADP generation in the suspension [13]; in contrast, under all circumstances respiration was dependent on ADP concentration through Michaelis-Menten kinetics with an apparent K_m of 17–25 μM [13]. In view of these observations, and correlation between the ADP levels and MVO_2 (fig.2), we suggest that in PG perfused hearts, mitochondrial respiration may be determined by ADP availability, and the coupling between MVO_2 and contractile activity is regulated by work dependent alterations in the steady-state concentration of myocardial 'free' ADP.

In G, or GI perfused hearts, the ADP content at all workloads exceeded the highest ADP level attained in the presence of pyruvate. Under these substrate conditions, a linear relationship between $[ADP]^{-1}$ and $(MVO_2)^{-1}$ was not evident. A consistent correlation between MVO_2 and the PP or the ATP/ADP ratio was also absent. During G perfusion, ATP/ADP ratio initially decreased from 180 $\pm$ 23 to a minimum of 94 $\pm$ 7 and subsequently increased with workload to 154 $\pm$ 16. This reversal of the ATP/ADP ratio was correlated with the onset of substantial consumption of endogenous lipid by these hearts as evidenced by experiments we performed in the presence of 13 μM 4-bromocrotonic acid, an inhibitor of fatty acid oxidation in perfused rat hearts [14]. Similarly, PP initially decreased and subsequently remained constant over the last four levels of increasing workload in G perfused hearts.

In the presence of insulin, ATP/ADP ratio remained relatively constant over the entire workload range; in this case, PP decreased with increasing workload. However, as in PG perfused hearts, all of the observed ATP/ADP ratios and the PP values should have been at one or the other extreme of the putative regulatory ranges of these parameters when compared to isolated mitochondrial data [13].

All of the above observations indicate the existence of a fundamentally different mechanism of respiratory control during glucose metabolism. Relevant to this issue, it was previously noted that there is a major difference between the metabolic states of glucose and pyruvate perfused hearts [1]. Even at very low workloads, levels of pyruvate, mitochondrial NADH, and tricarboxylic acid (TCA) cycle intermediates are at least an order of magnitude lower during GI perfusion relative to those obtained with PG as the substrate [1,16,17]. In the absence of insulin when glucose uptake rate is reduced, the relative depletion of the TCA cycle intermediates is expected to be even more pronounced. These observations suggest that under G or GI substrate conditions, carbon substrate delivery to the mitochondria is the rate-limiting step in mitochondrial NADH generation and ultimately in oxidative phosphorylation. The fact that inclusion of pyruvate in the perfusate leads to a large reduction in the cytosolic P_i and ADP content (table 1) supports this conclusion. This conclusion is also consistent with recent reports on isolated mitochondria where alterations in the oxygen consumption rate were achieved at a constant phosphorylation state by changes in carbon substrate metabolism [18], and on canine hearts in vivo where over the RPP range 5 to 25 $\times$ 10^3 mmHg/min, free ADP concentration was noted to be virtually constant [19]. The CP/ATP ratio of ~1.6 reported for the canine heart [19] is similar to the CP/ATP ratios we observed for the G and GI perfused hearts; in the RPP range corresponding to that encountered in the canine myocardium, the CP/ATP ratio was ~1.4 under G perfusion, and ~1.8 in GI perfused hearts. Over the entire RPP range examined by us (table 1) the CP/ATP ratio varied between 1.2 and 1.7 for G, 1.7 and 2 for GI and 2.1 and 2.2 for PG substrate conditions. Provided that other parameters that influence creatine kinase equilibria (such as intracellular pH and total creatine plus creatine phosphate content) were also similar, this suggests that the ADP level in the canine myocardium in vivo were high and its relative invariance with

workload in agreement with the data on G and GI perfused hearts. Furthermore, preliminary data we obtained on fatty acid perfused rat hearts ($n = 6$) showed that over the RPP range ~20 to ~95 × 10^3 mmHg both the CP/ATP ratio and ADP levels was approximately constant. The ADP level was comparable to that attained by pyruvate perfused hearts at the maximal workload (table 1); thus, although the ADP levels were low during fatty acid perfusion, they were not sufficiently low to become the rate limiting factor in respiration. The same is expected to be true in intact animals, since fatty acids are thought to be the dominant carbon source utilized by the myocardium in vivo.

It should be noted that for a given MVO_2, the rate of mitochondrial NADH synthesis and TCA cycle turnover must be the same under all of the different substrate conditions. However, this rate appears to be controlled by different processes in PG vs the G or GI perfused hearts. In the former case, the TCA cycle turnover appears to be determined by the utilization of its final product, i.e. mitochondrial NADH; this in turn is controlled by ADP availability to the mitochondria. Consequently, levels of NADH and TCA cycle metabolites are high. In the latter two cases, the carbon substrate delivery to the mitochondria appears to be the rate-determining process for mitochondrial NADH production and availability of mitochondrial NADH controls the rate of oxidative phosphorylation. This implies that in glucose perfused hearts, with or without insulin, workload-dependent control of the glycolytic rate [15] will be responsible for the coupling between MVO_2 and contractile activity in the domain where glucose can support the myocardial energy demand. Beyond this domain, the rate of mobilization and delivery of endogenous lipid to the mitochondria will also become regulatory.

In summary, the present data emphasize the importance of the carbon substrate available to the myocardium in determining the phosphorylation state, and definitively demonstrate that none of the three previously suggested parameters (ATP/ADP ratio, PP or ADP availability) is a universal regulator of mitochondrial respiration in the intact myocardium. Instead, the data suggest that the rate of oxidative phosphorylation may be governed by substrate limitation and that the rate limiting step in the chain of metabolic events involved in oxidative phosphorylation depends on the exogenous carbon source available to the myocardium.

ACKNOWLEDGEMENTS

Supported by NIH grants R01-HL33600 and 1K04-HL01241 and Veterans Administration Research Funds.

REFERENCES

[1] Kobayashi, K. and Neely, J.R. (1979) Circ. Res. 44, 166–175.
[2] Chance, B. and Williams, G.R. (1955) J. Biol. Chem. 217, 383–393.
[3] Slater, E.C., Rosing, J. and Mol, A. (1973) Biochim. Biophys. Acta 292, 534–553.
[4] Klingenberg, M. (1961) Biochem. Z. 335, 263–272.
[5] Holian, A., Owen, C.S. and Wilson, D.F. (1977) Arch. Biochem. Biophys. 181, 164–171.
[6] Nishiki, K., Erecinska, M. and Wilson, D.F. (1978) Am. J. Physiol. 234, C73–C81.
[7] Ugurbil, K., Petein, M., Maidan, R., Michurski, S. and From, A.H.L. (1986) Biochemistry 25, 100–107.
[8] Matthews, P.M., Williams, S.R., Seymour, A.M., Schwartz, A., Dube, G., Gaddian, D.G. and Radda, G.K. (1982) Biochim. Biophys. Acta 720, 163–171.
[9] Lawsen, J.W.R. and Veech, R.L. (1979) J. Biol. Chem. 254, 6528–6537.
[10] Garlick, P.B., Radda, G.K. and Seeley, P.J. (1982) Biochim. Biophys. Acta 720, 163–171.
[11] Williamson, J.R. (1966) J. Biol. Chem. 241, 5026.
[12] Morgan, H.E., Regen, D.M. and Park, C.R. (1964) J. Biol. Chem. 239, 369–374.
[13] Jacobus, W.E., Moreadith, R.W. and Vandegaer, K.M. (1982) J. Biol. Chem. 257, 2397–2402.
[14] Hutter, J.F., Schweikhardt, C., Piper, H.M. and Spieckermann, P.G. (1984) J. Mol. Cell. Cardiol. 16, 105–108.
[15] Neely, J.R., Liebermaster, H. and Morgan, H.E. (1967) Am. J. Physiol. 212, 815–822.
[16] Williamson, J.R. (1965) J. Biol. Chem. 240, 2308–2321.
[17] Chapman, J.B. (1972) J. Gen. Physiol. 59, 135–154.
[18] Koretsky, A.P. and Balaban, R.S. (1986) Biophys. J. 49, 207a.
[19] Balaban, R.S., Kantor, H.L., Katz, L.A. and Briggs, R.W. (1986) Science 232, 1121–1123.