15 Top down analysis of heart bioenergetics

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Introduction

Skeletal muscle bioenergetics are characterized by an energy balance maintained despite a 30- to 40-fold range of ATP utilization [1]. This energy balance—the magnitude of ATP generation meets ATP utilization—is achieved with improved homeostasis of energetic intermediates, which is even more pronounced in heart as compared to skeletal muscle. How this homeostasis is achieved is still unclear and the distribution of control has still not been fully described.

Perfused rat heart therefore appears as an interesting system for the application of top-down analysis [2] to muscle bioenergetics. This approach should allow the measurement of the control distribution between energy producers and consumers under the different working conditions chosen for this study. The access to the elasticities of the different subsystems seems even more promising for the study of regulations or dysfunctions [3,4].

Methods

Experiments were carried out on Langendorff-perfused isolated rat heart, as previously described [5]. Isovolumetric contractile activity was assessed using a balloon inserted in the left ventricule connected to a pressure sensor and a recorder. For accuracy, oxygen consumption measurements were carried out outside the magnet under identical perfusion conditions. ^{31}P NMR spectra were recorded using a 20 mm tube inserted in a 9.7T magnet [4].

The preliminary scheme for top-down analysis application to heart bioenergetics is presented in Fig. 15.1. For this study, all intermediates (NMR) concentrations were considered and the system was therefore studied as two subsystems linked

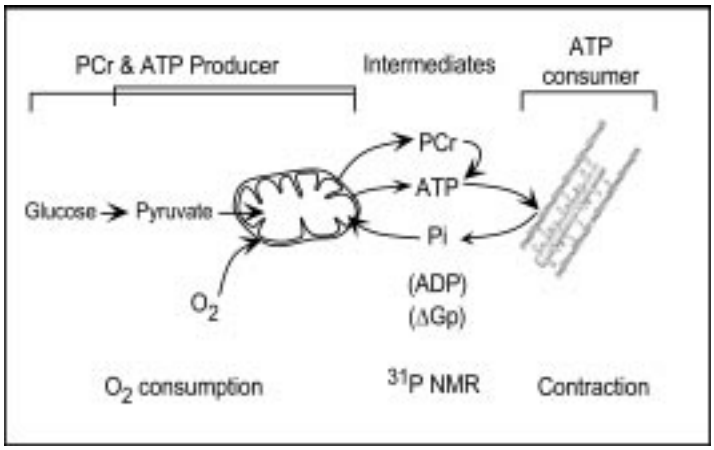

Fig. 15.1 Definition of the subsystems for top-down analysis of perfused heart. The Producer comprises mitochondria and all the steps from substrate supply. The producer is therefore diffferent for pyruvate or glucose. The Intermediates which are not directly measured by NMR are in parenthesis. The ATP consumer comprises all the ATP utilization linked to contractile activity.

by several intermediates. With working heart, ATP consumer subsystem is mainly due to contraction (myosin ATPases and calcium re-uptake by sarcoplasmic reticulum). We studied two different substrates (glucose and pyruvate) and therefore the ATP/PCr producer, which comprises all the steps form substrate supply to mitochondria, varies with the substrate used. For each substrate two different calcium concentrations were used in the perfusion medium in order to modulate cardiac activity.

Results and discussion

Substrate and calcium modulations of perfused heart activity

The relationship between oxygen consumption of the heart and the developed RPP (rate pressure product) is presented in Fig. 15.2. Results obtained after KCN additions (used for determination of elasticities, see below) were added to this graph to show the absence of modification of the system under these conditions. Under low calcium conditions (2 mM), both substrates gave the same mechanical performance. By contrast, when the calcium concentration was increased to 3.5 mM, the heart showed a much higher activation when pyruvate was used, although the relationship between RPP and oxygen consumption was conserved. This relationship appeared linear and presented an intercept corresponding to a very low oxygen consumption, suggesting that the yield of heart contraction is constant over a important range of activity.

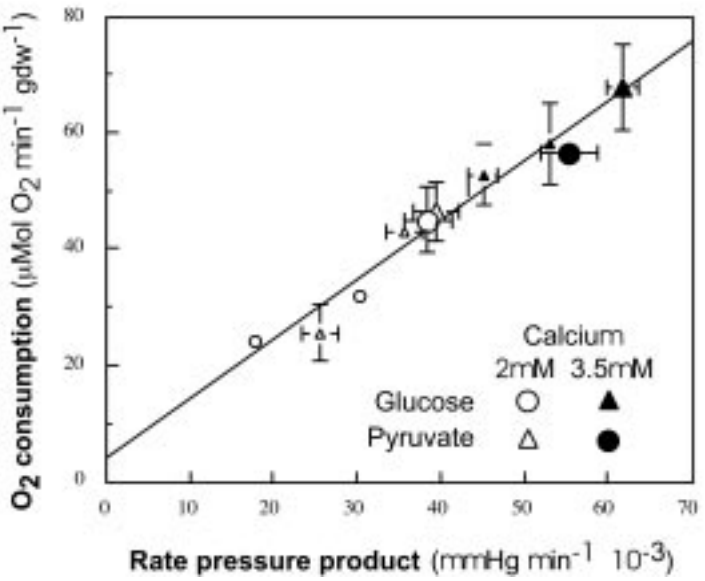

Fig. 15.2 Relationship between the rate pressure product (RPP) and the oxygen consumption of perfused rat heart under the different conditions of perfusion. The smaller symbols refer to KCN inhibition (see text).

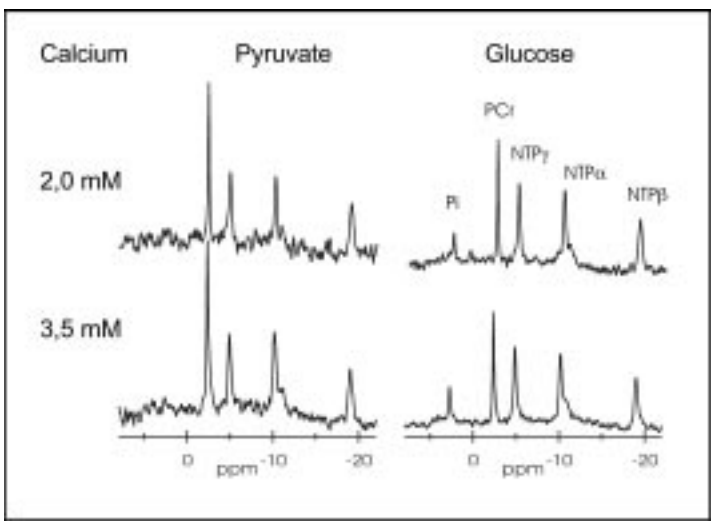

Fig. 15.3 ^{31}P NMR spectra of perfused rat heart under the different conditions of perfusion

Table 15.1 Elasticity of ATP consumer towards PCr concentration under the different substrate and calcium conditions. Errors are expressed as SD for 5 independent experiments for each condition. Difference is significant for 3.5 mM Ca^{++} ($p < 0.005$), it may be noted that significant difference is observed under both calcium conditions for ΔGp (not shown).

Calcium (mM)	Glucose	Pyruvate
2.0	0.51 ± 0.15	0.73 ± 0.22
3.5	0.38 ± 0.11	0.63 ± 0.03

How about the intermediates? ^{31}P NMR spectra recorded under the four different perfusion conditions are presented in Fig. 15.3. The first observation is that, although the activity was identical for both substrates in the presence of 2 mM Ca^{2+} (see Fig. 15.1), the intermediates concentrations were different. While ATP concentration was always constant, phosphate (Pi) increased and phosphocreatine (PCr) decreased when glucose was used. Spectra were not linked to heart activity: almost no change in the spectra was observed when $Ca^{2+}$was increased (Fig. 15.3). It is interesting to note that homeostasis of all intermediates was achieved also for glucose although the heart failed to increase its activity at the same level as obtained with pyruvate.

Top down analysis

At this stage of the study, only the elasticities of the Consumer subsystem towards the different intermediates have been characterized under the different working conditions described. Following the principles of top-down analysis, this was carried out by inducing relatively small changes in the intermediates and measuring the relative changes in intermediates concentrations and flux (oxygen consumption and contraction). KCN (0.1–0.2 mM) was used after verification of the absence of side effect (see Fig. 15.2). Under these conditions, ATP was not affected by KCN inhibition while PCr was decreased and Pi increased (the sum PCr+Pi was unchanged). Except for ATP, the elasticities towards the different intermediates (including ΔGp calculated from creatine-kinase equilibrium) evolved the same way, since all these intermediates are linked. Therefore, as a first approximation it seems reasonable to use PCr, which is directly accessible from NMR experiments (Table 15.1).

It is hazardous to try to deduce too much from the data obtained so far. However, it seems possible to draw some lines from this study. First, Table 15.1 shows that the elasticity of the contraction measured in the presence of glucose is systematically lower than that observed using pyruvate under the same calcium concentration. This result suggests that control by contraction may be lower (if parallel modifications occur for the producer) in the presence of glucose and correlate

with the addition of enzymatic processes to the producer when glucose is used instead of pyruvate (see Fig. 15.1). The second observation is that the increase in calcium concentration and heart activity seems to induce a decrease in the elasticity of the contraction, and therefore an increase in the control by contraction (if parallel . . ., see above). This result could be interpreted as an indication for a higher activation of the producer (mitochondrial) than of the consumer (contraction).

Conclusion

This study represents the first part of the top-down analysis of heart bioenergetics. Some conclusions may however be drawn with caution, keeping in mind that the full analysis requires measurement of the elasticities of the producer towards the adequate intermediate, likely the energy charge [6]. Comparison between glucose and pyruvate as substrate suggests that modifying the energy producer by additional steps may increase the control exerted by that subsystem. Therefore, at least in the case of glucose, the control appears distributed between the consumer and the producer. The homeostasis of the intermediates for a wide range of cardiac activity may be explained by a simultaneous modulation of both producer and consumer by calcium.

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