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Non-invasive integrative analysis of contraction energetics in intact beating heart☆

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ABSTRACT

The comprehensive study of human pathologies has revealed the complexity of the interactions involved in cardiovascular physiology. The recent validation of system's biology approaches – like our Modular Control and Regulation Analysis (MoCA) – motivates the current interest for new integrative and non-invasive analyses that could be used for medical study of human heart contraction energetics.

By considering heart energetics as a supply–demand system, MoCA gives access to integrated organ function and brings out a new type of information, the "elasticities", which describe *in situ* the regulation of both energy demand and supply by cellular energetic status. These regulations determine the internal control of contraction energetics and may therefore be a key to the understanding of the links between molecular events in pathologies and whole organ function/dysfunction. A wider application to the effects of cardiac drugs in conjunction with the direct study of heart pathologies may be considered in the near future. MoCA can potentially be used not only to detect the origin of the defects associated with the pathology (elasticity analyses), but also to provide a quantitative description of how these defects influence global heart function (regulation analysis) and therefore open new therapeutic perspectives. Several key examples of current applications to intact isolated beating heart are presented in this paper. The future application to human pathologies will require the use of non-invasive NMR techniques for the simultaneous measurement of energy status (^{31}P NMR) and heart contractile activity (3D MRI).

This article is part of a Directed Issue entitled: Bioenergetic dysfunction, adaptation and therapy.

1. Introduction

Cardiovascular disease is the first cause of mortality in the whole world, accounting for 29% of deaths, followed by infectious diseases (16.2%) and cancers (12.6%). Cardiac-specific diseases are responsible for 700 000 deaths each year in Europe. Half of this mortality is due to heart failure (ineffective contraction) which is a chronic process. The impact of cardiac diseases of genetic origin is relatively low, whereas prevalence of acquired modifications of tissue substrate (ischemic events, infarction scar, cardiomyopathy or aging) is essential. Cardiac contraction is entirely dependent on electrical activation, an important part of heart failure cases is secondary or aggravated by electrical dysfunctions, potentially reversible by targeted therapies: ventricular dyssynchrony present in >50% of such patients, and atrial fibrillation present in 24% of them, and also the main cause of cerebral embolic strokes. However, cardiac contraction is also entirely dependent, downstream of the electrical stimulation on the adequacy in the response of contractile apparatus and in energy balance, both disrupted during heart failure.

Cardiovascular disease, and the cardiovascular side effects of drugs, are essentially multifactorial problems involving interactions between many proteins, dependent on highly organized cell, tissue and organ structures. This is one reason why the side effects

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of drugs are often unanticipated. It is impossible to unravel such problems without using a systems approach, *i.e.* focusing on processes, not just molecular components (Fink and Noble, 2010). As a consequence, biochemical modifications of heart function must reflect this complexity. It is also more and more obvious that fine molecular analyses of pathologies should be fruitfully combined with integrative approaches, since integrated organ function reveals fundamental properties that make it different than the sum of the underlying molecular events. Therefore, it appears that an important issue in the comprehension of the link between molecular events in pathologies and whole organ function/dysfunction is the development of new experimental strategies aimed at the study of integrated organ physiology.

The complete description of a biological or physiological function depends on the synergy between adapted experimental and analytical approaches. System's biology of important biological functions, like heart contraction, is a current issue in physiology as it not only reveals a new type of fundamental informations, but also may turns out as new diagnostic and therapeutic strategies (Cascante et al., 2002). System's Biology starts with what we have learned from the huge progress at the molecular level and goes further toward understanding of how small scale events integrate into biological functions. These new strategies require both the development of non-invasive techniques allowing investigations at the integrated level (*ex vivo* and *in vivo*) and suitable analytical tools. The analytical tools of the Top-down or Modular approaches to Metabolic Control Analysis (MCA) (Fell, 1992; Heinrich and Rapoport, 1974; Kacser and Burns, 1973, 1995) were used by us and others to overcome the complexity of intra-cellular regulations (Brand, 1996; Brand and Curtis, 2002; Brown et al., 1990; Dufour et al., 1996; Hafner et al., 1990) and to describe heart energetics (Diolez et al., 2007, 2002). In the Top-down (modular) approach, the complexity of the system is reduced by grouping reactions and reactants into large modules connected by a small number of intermediates. While each module may be of any complexity, their interactions should only take place through the identified intermediate(s). The fundamental principle is that the kinetic interactions (elasticities) (Fell, 1992; Heinrich and Rapoport, 1974; Kacser and Burns, 1973, 1995) between the intermediates (substrates and products) and the different modules (or single enzymes) determine and maintain steady state conditions (fluxes and concentrations). Therefore regulation of the pathways (*i.e.* activity changes) involves modulations of these kinetic interactions that can be studied by MCA.

2. Modular Control and Regulation Analysis (MoCA) of heart energetics

By combining MCA with non-invasive ^{31}P NMR measurement of energetic intermediates and simultaneous measurement of heart contractile activity, we developed MoCA (Modular Control and Regulation Analysis), an integrative approach to study *in situ* control and regulation of cardiac energetics during contraction in intact beating perfused isolated heart (Diolez et al., 2007). Like muscle and brain, other energy consuming organs, heart tissues contain apart from ATP a second energy-transferring molecule: phosphocreatine (PCr). MoCA describes the energetics of heart contraction as a two-module system (energy Supply and energy Demand) connected by the energetic intermediates ATP and PCr (Fig. 1). The elasticities (responsiveness) of Supply and Demand modules to PCr changes may be experimentally determined in intact beating heart and further used to describe normal or pathological control pattern and perform regulation analysis of pathologies and drugs (see Diolez et al., 2007, for a complete description of the analysis).

As a major outcome, this integrative approach allows to measure and compare the relative importance of the different routes – *i.e.* supply, demand and metabolite changes – by which the effect(s) of a drug/hormone (Korzeniewski et al., 2008, 2009; Deschodt-Arsac et al., 2010b) or a pathology (Calmettes et al., 2008, 2010) are transmitted and alter integrated organ function, *e.g.* how inotropic drugs/action activates heart contraction. The complete description of MoCA and the possible use can be found elsewhere (Deschodt-Arsac et al., 2010a; Diolez et al., 2007) and we will present in the present paper the applications to various fundamental questions regarding regulation of heart energetics.

3. Physiological activation of heart contraction

3.1. Energetics of heart contraction

By contrast with skeletal muscle, where PCr and phosphorylation potential (ΔGp) decrease during contraction, the energy balance in heart bioenergetics is characterized by an improved homeostasis (Balaban, 2002; Balaban et al., 2003; Kushmerick, 1995). Indeed, almost no changes in the energetic intermediates (PCr, ATP and Pi) are observed following important increase in heart activity, as seen for instance by ^{31}P NMR spectroscopy on isolated perfused heart (Diolez et al., 2000, 2007). How this homeostasis is achieved in heart – but not in muscle – during the so-called excitation–contraction coupling is still a debated fundamental question.

Present in high concentration in myocytes, PCr may now be considered as being directly produced by mitochondria from free cytosolic creatin and therefore plays a central buffering role in energy transfer in muscle (Fig. 2). The essential role of calcium in the coupling process from electrical excitation of the myocyte to contraction in heart is now well established and appears as a good lead to a better understanding of excitation–contraction coupling (Balaban, 2002; Balaban et al., 2003). Ca^{2+} is the direct activator of the myofilaments, and therefore contraction depends on free intra-cellular calcium concentration ($[Ca^{2+}]_i$). Indeed, myocyte mishandling of Ca^{2+} is a central cause of contractile

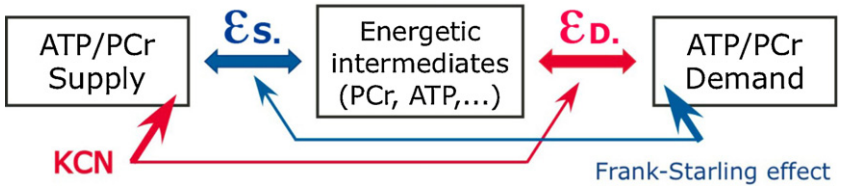

Fig. 1. System definition for MoCA of the energetics of perfused heart (figure from Deschodt-Arsac et al., 2010a). The system of contraction is defined as two modules connected by the pool of energetic intermediates. The Supply module comprises all the metabolic steps from substrate supply (including oxygen supply) to mitochondrial production of PCr. The Demand module comprises all the steps consuming ATP/PCr linked to contractile activity (contraction of myofilaments and ATP used for Ca^{2+} recapture). The elasticities of each module toward the intermediates (PCr measured by ^{31}P NMR is considered as representative) are calculated after inducing slight changes in steady state contraction by alteration of the other module. Cyanide (KCN) was chosen to specifically inhibit mitochondrial cytochrome oxidase and measure Demand elasticity (ε^{D}_{PCr}) as the response of the system to the relative PCr concentration change observed. On the other hand, change in internal balloon pressure (Frank–Starling effect) was used to increase contractility and measure Supply elasticity (ε^{S}_{PCr}) (see Diolez et al., 2007).