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Modular regulation analysis of heart contraction: application to in situ demonstration of a direct mitochondrial activation by calcium in beating heart

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Diolez P, Deschotd-Arsac V, Raffard G, Simon C, Dos Santos P, Thiaudière E, Arsac L, Franconi J-M. Modular regulation analysis of heart contraction: application to in situ demonstration of a direct mitochondrial activation by calcium in beating heart. Am J Physiol Regul Integr Comp Physiol 293: R13–R19, 2007. First published June 1, 2006; doi:10.1152/ajpregu.00189.2006.—Heart contraction is characterized by the absence of changes in energetic intermediates in response to a large increase of activity. Until now no experimental approach could address this question concerning the intact beating heart. Ca2+ plays a crucial role in the excitation-contraction coupling, and in vitro studies have evidenced that Ca2+ may also directly activate mitochondrial oxidative phosphorylation. We applied our new in situ modular control and regulation analysis on isolated beating rat heart perfused under two different calcium concentrations with pyruvate or glucose as the substrate. Modular control analysis demonstrated experimentally that, although control by energy production was slightly higher under glucose conditions compared with pyruvate, most of the control of heart contraction resides in energy utilization. This behavior is the direct consequence of the high sensitivity (elasticity) of the energy producer processes to ATP utilization. Interestingly, the increase in heart metabolic rate by Ca2+ did not significantly change the pattern of control distribution. The regulation analysis performed under the two calcium conditions demonstrated a balanced activation of myofibrils ATPases, and mitochondrial ATP synthesis in response to Ca2+ increase. This first study demonstrates in situ the hypothesis that the energetic adequation in heart contraction is mediated by a parallel activation of both processes of energy production and utilization by Ca2+. The results presented here show that modular control and regulation analyses allow in situ study of internal regulations in intact beating heart energetics and function and may now be applied to heart dysfunctions and therapeutic effects.

rat perfused heart; 31P-labeled magnetic resonance spectroscopy; calcium signaling; metabolic control analysis; systems biology

LIKE MUSCLE AND BRAIN, heart tissues contain, apart from ATP, a second energy-transferring molecule, phosphocreatine (PCr). Present in high concentration, PCr may now be considered as being directly produced by mitochondria from free cytosolic creatine and therefore plays a central buffering role in energy transfer in muscle. However, by contrast with skeletal muscle, the energy balance in heart bioenergetics is characterized by an increased homeostasis (3, 4, 6, 30). Indeed, only slight changes in the energetic intermediates (PCr, ATP, and P) are observed following important increases in heart activity, as seen, for instance, by 31P-labeled NMR on isolated perfused heart (13). How this homeostasis is achieved in heart but not in muscle during the so-called excitation-contraction coupling is still unknown.

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 (3, 5, 27). Ca2+ is the direct activator of the myofilaments, and therefore contraction depends on free intracellular calcium concentration ([Ca2+]i). Indeed, myocyte mishandling of Ca2+ is a central cause of contractile dysfunctions (7). In heart, rhythmic contraction is the consequence of transient changes in [Ca2+]i. Furthermore, there is now increasing evidence that Ca2+ has multiple effects on other intracellular processes and particularly on several mitochondrial functions (19, 23, 32), including dehydrogenases, ATP synthase, and, more recently, the binding of PCr-kinase with mitochondrial membranes (34). Thus, activation of mitochondrial oxidative phosphorylation during Ca2+ transient may now also be considered as an important mechanism in excitation-contraction coupling in heart (25–27) (Fig. 1). Therefore, in heart, the electrical excitation of the myocyte may result in a parallel activation by Ca2+ of both energy-producing and energy-consuming processes (3, 5, 12, 13, 25–27). This mechanism would be fundamental in the capacity for the heart to dramatically increase contraction without any change in energetic intermediates. Until now, while theoretical studies have demonstrated the necessity for such parallel activation to occur in heart contraction (25–27), no experimental evidence could be obtained. One of the challenges for the future is to develop integrated in situ experimental approaches to evaluate such complex biological processes and to lead to a comprehensive understanding of basic cellular properties (3).

The new tools of the top-down or modular approaches to metabolic control analysis were used by us and others to overcome the complexity of intracellular regulations (9–11, 15, 18) and to describe heart energetics (12). Metabolic control analysis (16, 20–22) can effectively be used to describe and quantify controls and regulations in complex systems (10). In
the top-down 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 (16, 20 – 22)] between the intermediates (substrates and products) and the different modules (or single enzymes) determine and maintain steady-state conditions (fluxes and concentrations).

Experimentally, the full analysis requires the measurement of all fluxes and intermediates involved. Since our main objective was to develop potential therapeutic applications, we decided to address this crucial point in situ and noninvasively on intact beating heart: the Langendorff-perfused rat heart (see EXPERIMENTAL PROCEDURES). We previously described the first application to intact heart (12) in which we defined two modules: the producer and the consumer of the energetic intermediates (Fig. 2). Energetic intermediates in heart and muscle are phosphorylated molecules (PCr, ATP, ADP, and P) that could be kinetically studied using 31P-labeled NMR (12), whereas energy flux could be estimated by measuring heart contractile activity (see EXPERIMENTAL PROCEDURES) and/or total heart oxygen consumption (12). In working heart, the ATP consumer module comprises all the ATP consuming processes occurring during contraction (mainly myosin ATPases and calcium reuptake by sarcoplasmic reticulum and cell membrane). As for the producer, we used two different substrates (glucose and pyruvate) with the consequence of studying two different modules, including glycolysis enzymes and mitochondria (with glucose in the absence of pyruvate) or only mitochondria (in the presence of pyruvate, directly oxidizable by mitochondria).

As achieved here on the role of cytosolic Ca2+ in excitation-contraction coupling, the exact targets of any effector of heart contraction may be studied through the approach proposed in Regulation analysis. Whereas modular control analysis (MoCA) describes how steady states are maintained (flux and metabolites homeostasis, see above), regulation analysis (8, 10) has the potential to provide a quantitative description of how regulation by an external or internal effector leads the system to move to a new steady state. In the present study, we were able to measure and compare the relative importance of the different routes within the heart by which the effects of Ca2+ are transmitted.

**EXPERIMENTAL PROCEDURES**

**Langendorff Perfusion and NMR Measurements**

Male Sprague-Dawley rats (350 – 400 g) were used. Rats were anesthetized with 40 mg of pentobarbital injected intraperitoneally. The thorax was opened, and hearts were rapidly excised, immediately cooled in iced Krebs buffer, and perfused by an aortic cannula at constant pressure (100 mmHg) with Krebs-Henseleit solution (95% O2-5% CO2, pH 7.35, temperature 37°C) perfused using a solution containing 10 mmol/l Na-pyruvate or 16.7 mmol/l glucose as substrate, as described previously (14). Depending on the experiments, actual free calcium concentration was set to 2.0 or 3.5 mmol/l. Left ventricle systolic pressure of unpaced heart was measured under isovolumic conditions of contraction by a latex balloon inserted into the left ventricle and connected to a pressure transducer. Mechanical performance was evaluated as the product of heart frequency and developed pressure [rate-ventricular pressure product (RPP) in mmHg·beat·min−1]. The perfused heart was placed into a 20-mm NMR tube inserted into a 9.4-T super-conducting magnet equipped with a 20-mm bore (Bruker DPX400 Avance) for 31P-labeled NMR spectroscopy. To eliminate endogenous substrates and remaining anesthetics, 20 min were allowed for stabilization of heart activity before any measurement was carried out (28, 29). Except for specific modifications for MoCA application (see below), all experimental procedures have been described previously (14).

For the study of steady-state modifications in the framework of MoCA, four NMR spectra (5-min duration time for each spectrum) were acquired before modulation was applied, then after a transition time equivalent to one spectrum (5 min) four new spectra (20 min) were recorded. Each steady state (before and after modulation) was characterized by the mean values of the four spectra for RPP and phosphorylated compounds.

**MoCA**

We previously described our new approach of intact perfused heart energetics using MoCA (12). By using this approach, complex sys-
tems may be simplified by grouping reactions and reactants into large modules connected by a small number of explicit intermediates (10). We applied this to the study of energy transfer during contraction in heart (or muscle) by defining two modules (so-called producer and consumer) linked by several obligatory energetic intermediates (see Fig. 2). In working heart, as ATP concentration was unchanged under all physiological conditions, PCr was chosen as the representative intermediate between energy production and consumption (12). The producer module is then defined as all the steps from substrate and oxygen supply to mitochondrial PCr production through the PCr-CK system (Fig. 1), therefore it includes glycolysis when glucose is used as substrate instead of pyruvate directly oxidized by mitochondria. The consumer module comprises all the ATP consuming processes occurring during contraction (myosin ATPases and calcium reuptake by sarcoplasmic reticulum and cell membrane) (12). Control and regulation of our modular system can be analyzed using MoCA, as long as intermediate concentrations and module activities (flux) can be studied. Continuous 31P-labeled NMR spectroscopy gives access to all energetic intermediates, including our intermediate PCr. The energetic flux through the system was simultaneously assessed as the contractile activity measured by heart RPP (see above).

Control analysis. Following the principles of top-down analysis, control coefficients over the energy flux in heart contraction were calculated from the overall elasticities of the producer and consumer modules toward the intermediate PCr (9). In this study, the elasticity (ε_module) of each module toward the intermediate may be calculated from the changes in flux (RPP) and PCr concentration ([PCr]) induced by any slight modulation of the other module (see Ref. 12 for details). Experimentally, to measure consumer elasticity (ε_C), two low concentrations of cyanide (0.1 and 0.2 mM KCN) were used to progressively inhibit mitochondrial cytochrome oxidase. Then the evolution in RPP was plotted as a function of [PCr], and ε_C was calculated from the relative slope of this curve

$$\varepsilon_{\text{Module}} = \frac{\text{ΔRPP/RPP}}{\text{PCr}}$$

Producer elasticity ($\varepsilon^p_{\text{PCr}}$) was measured the same way, but changes in [PCr] and RPP were induced by increasing internal balloon volume (from 100 to 150 μl) to trigger increased heart contractility. The elasticity was calculated by using the previous equation.

Elasticities were calculated for each heart, and results are expressed as the mean value of all experiments (see Table 2). The control coefficients of both modules ($C^{\text{Pro}}$ and $C^{\text{Cons}}$) were thus calculated from experimentally measured $\varepsilon^p_{\text{PCr}}$ and $\varepsilon^c_{\text{PCr}}$, according to summation and connectivity theorems (21).

Regulation analysis. The rationale for regulation analysis can be found elsewhere (2, 8). We will only detail here our application to modular regulation analysis of heart energetics.

As for any effector, data required for the complete regulation analysis of the effect of increasing calcium concentration from 2 to 3.5 mM are 1) the complete control analysis at low calcium concentration (elasticities toward the intermediates and flux control coefficients) corresponding to the starting conditions; and 2) the global effect of calcium increase on the flux and the intermediates expressed as relative changes in RPP and [PCr].

The direct effect of calcium increase on each Module is different from the total measured effect in that module activity is also affected by indirect changes in the intermediate PCr due to concomitant modification of the activity of the other module. For each module, the indirect effect due to this relative change in PCr may be easily deduced from elasticity analysis; it is the product of the relative change in PCr and the module’s elasticity toward PCr.

Indirect Effect on Producer:  
$$\varepsilon^p_{\text{PCr}} \times \Delta[\text{PCr}]_{C2^-}/[\text{PCr}]$$

Indirect Effect on Consumer:  
$$\varepsilon^c_{\text{PCr}} \times \Delta[\text{PCr}]_{C2^-}/[\text{PCr}]$$

Therefore, the direct effect of calcium on each module [defined as the integrated elasticity (IE)] (2, 8) is calculated as the difference between the total measured effect of the calcium increase on contraction (ΔRPP/C2\textsuperscript{-}/RPP) and the previously calculated indirect effect.

Direct Effect on Producer:  
$$\text{IE}^p_{\text{C2^-}} = \frac{\Delta[\text{PCr}]_{C2^-}}{[\text{PCr}]_{C2^-}} \times \Delta[\text{PCr}]_{C2^-}/[\text{PCr}]$$

Direct Effect on Consumer:  
$$\text{IE}^c_{\text{C2^-}} = \frac{\Delta[\text{PCr}]_{C2^-}}{[\text{PCr}]_{C2^-}} \times \Delta[\text{PCr}]_{C2^-}/[\text{PCr}]$$

The final important parameter for the description of the regulation of heart contraction by calcium is the global response (IR) (2, 8), which describes quantitatively how activation of each module by calcium influences total heart inotropic response. By definition, the control coefficient links the change in module activity to the change in the flux. Therefore, the integrated response for each module is the product of the control coefficient and the direct effect on this very module.

Global Response through Producer:  
$$\text{IR}^p_{\text{C2^-}} = \frac{\Delta[\text{PCr}]_{C2^-}}{[\text{PCr}]_{C2^-}} \times \Delta[\text{PCr}]_{C2^-}/[\text{PCr}]$$

Global Response through Consumer:  
$$\text{IR}^c_{\text{C2^-}} = \frac{\Delta[\text{PCr}]_{C2^-}}{[\text{PCr}]_{C2^-}} \times \Delta[\text{PCr}]_{C2^-}/[\text{PCr}]$$

Statistical Analysis

Experimental values are reported as means ± SD for the number of independent rat hearts. Statistical comparisons between groups (glucose vs. pyruvate, high vs. low calcium concentrations) were performed by ANOVA. The error associated with calculated control coefficients (C), IE, and responses coefficients (IR) was determined by using Monte Carlo simulations (1). Experimental values of ΔPcr/PCr and ΔRPP/RPP were simulated from observed means and SDs. Simulations were based on normal distribution with a mean equal to the mean of the experimental points and an SD equal to the SD of the experimental points. ε, C, IE, and IR were calculated as described in above sections from simulated values.

RESULTS AND DISCUSSION

MoCA

There are two ways of modulating the strength of cardiac contraction: by altering the amplitude or duration of the cytosolic Ca2\textsuperscript{+} transient, and by altering the sensitivity of the myofilaments to calcium (7). Experimentally, the classical way to modulate cytosolic Ca2\textsuperscript{+} transients is to change the calcium concentration in the perfusate, which induces different contractile activities of isolated perfused heart. It has also been shown that myocardial oxygen consumption and contractile activity are proportional to cytosolic Ca2\textsuperscript{+} concentration (3, 35). We have previously shown that under our experimental conditions (2 mM and 3.5 mM Ca2\textsuperscript{+}) the contractile activity was proportional to the oxygen consumption of the heart, suggesting a constant energetic efficiency of heart contraction over a large range of activity (12). Therefore, there is a linear relationship between Ca2\textsuperscript{+} concentration in the perfusate and cytosolic [Ca2\textsuperscript{+}] (3), and by using these two different Ca2\textsuperscript{+} concentrations, we determined two different steady-state levels of heart activities characterized by two cytosolic [Ca2\textsuperscript{+}]. The comparison of these two conditions by applying MoCA allows the quantitative description of the effect of cytosolic Ca2\textsuperscript{+} on heart contraction.

Table 1 presents the physiological state of the perfused rat hearts under the four conditions studied here. Main observations deal with the classical decreased PCr concentration when
glucose was used as substrate associated with an increase in free Pi concentration (not shown). The first step in the analysis is the determination of the kinetic interactions (elasticities) of the two modules to PCr concentration or, in other words, how each module responds to small changes in PCr (see EXPERIMENTAL PROCEDURES). These changes (not physiologically occurring in heart) were artificially provoked by inhibiting or activating the other module (see Fig. 2). To determine producer response to PCr changes, myofilament Ca\(^{2+}\) sensitivity (consumer) was enhanced dynamically by stretching slightly the myofilaments \(\times \) (Fig. 3A). This was carried out by increasing the volume of the balloon inserted in the left ventricle (as when the heart fills with blood) otherwise used to continuously measure heart contractile activity. The resulting stronger contraction is mainly due to the transverse filament lattice compression that occurs on stretch, which enhances the actin-myosin interaction \((7, 17)\). This is the mechanism by which the heart adjusts to diastolic filling, i.e., the Frank-Starling response. It has been shown that the rapid increase in twitch force after muscle stretch was not associated with a rise in calcium transient \((24, 31)\) or a modification of calcium recirculation \((33)\). In this study, the experiment was started under low pressure conditions, and the balloon was further inflated to normal pressure. This increase in balloon pressure induced a small but measurable change in PCr concentration \((\text{Fig. 3A})\) associated with an increase in heart contractile activity, reflecting the response of the producer module to the induced change in PCr \((\text{Table 2})\). As to the producer, as any specific mitochondrial inhibitor will do, cyanide (at very low concentration) was chosen for this study to determine consumer elasticity to PCr \((\text{Fig. 3B})\). Under all PCr modulation experiments, the absence of any shift of Pi peak indicated the absence of pH change.

Table 2 presents all the experimental data required for the complete description of the internal effect of Ca\(^{2+}\) on heart contraction. First, the inotropic response of beating heart to Ca\(^{2+}\) increase was higher in the presence of pyruvate than in the presence of glucose \((36.8 \text{ vs. } 19.4\%\text{, } P < 0.05)\), and almost no change in PCr was observed in these experiments under both substrates conditions \((\pm 0.7\%\text{ vs. } -2.8\%\text{ for pyruvate and glucose, respectively})\). Table 2 also presents the changes in contractile activity induced by experimental modulations of PCr obtained either by cyanide inhibition of mitochondria or by modulation of balloon pressure. We could verify that the low KCN concentration used did not induce any change in ATP concentration (not shown, see Fig. 3). \(^{31}\)P-labeled NMR allowed the calculation of the changes in phosphate potential \((\Delta \text{Gp})\) induced during these experiments and showed that \(\Delta \text{Gp}\) was modified by < 1\% (results not shown). Altogether, these results indicate that, under our conditions, the effect of KCN was strictly on PCr production, and the absence of modification of AGp indicates that ATP consuming processes \((\text{e.g., sarco-plasmic reticulum } Ca^{2+}\text{ handling})\) were not affected. From Table 2, it can be seen that when the decrease in PCr was induced by inhibition of the producer module by cyanide, a comparable decrease in both PCr and contractile activity was observed. By contrast, when balloon pressure was increased, an important increase in contractile activity was associated with only a small change in PCr. These results demonstrate that producer (mainly mitochondria) is highly sensitive to changes in PCr concentration by comparison with consumer (myofilaments).

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**Fig. 3.** Typical changes in \(^{31}\)P-labeled NMR spectra of perfused rat liver induced by increase in balloon pressure from low to normal \((A)\) and addition of 0.1 mM KCN to perfusate \((B)\). On this representative experiment, isolated rat hearts were perfused using pyruvate as substrate and in the presence of 3.5 mM calcium. NTP, nucleoside triphosphate \((\text{essentially ATP})\); \(\alpha, \beta, \text{ and } \gamma\), position of the three phosphate atoms; PCr, phosphocreatine; Pi, inorganic phosphate.
Table 2. Relative changes in PCr and in contractile activity induced by a small increase in intraventricular pressure (activity increase) or by mild mitochondrial inhibition by cyanide.

<table>
<thead>
<tr>
<th>Ca(^{2+})</th>
<th>2.0 mM</th>
<th>Ca(^{2+})</th>
<th>3.5 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pyruvate medium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. independent experiments</td>
<td>11</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>PCr ((\Delta\text{PCr}/\text{PCr}_i))</td>
<td>+0.7±0.1</td>
<td>−5.2±2.2 †</td>
<td>−24.9±3.1</td>
</tr>
<tr>
<td>Contractile activity ((\Delta\text{RPP}/\text{RPP}_i))</td>
<td>+36.8±2.8 †</td>
<td>+58.0±13.2</td>
<td>−14.1±3.2</td>
</tr>
<tr>
<td><strong>Glucose medium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. independent experiments</td>
<td>12</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>PCr ((\Delta\text{PCr}/\text{PCr}_i))</td>
<td>−2.8±0.3</td>
<td>−14.9±3.9 †</td>
<td>−51.0±6.2</td>
</tr>
<tr>
<td>Contractile activity ((\Delta\text{RPP}/\text{RPP}_i))</td>
<td>+19.4±3.1 †</td>
<td>+79.5±13.2</td>
<td>−52.0±18.1</td>
</tr>
</tbody>
</table>

Data are compared with changes observed during calcium activation of contraction. *P < 0.01 between glucose and pyruvate medium; †P < 0.05 at high calcium concentration with glucose and pyruvate, respectively. While control by energy production was always low, it was doubled under glucose conditions compared with pyruvate (21.2% compared with 2.3% in glucose and 3.0% in pyruvate medium).

These experimental data were used to calculate the MoCA coefficients (see EXPERIMENTAL PROCEDURES) reported in Table 3. The high sensitivity of the producer is now expressed as an important absolute value of the elasticity coefficient, and the converse is true for the consumer. The constraint introduced by using glucose as substrate instead of pyruvate resulted in a significant decrease in the elasticity (sensitivity) of the producer to changes in PCr.

Calculated control coefficients represent the percentage of the control exerted by each module on the overall contractile activity of beating heart (Table 3). Results indicate that under our conditions, most of the control of contraction resides on energy utilization (78.8% with glucose compared with 92.3% with pyruvate \(P < 0.05\) at low calcium, and 85.1% compared with 96.0% \(P < 0.05\) at high calcium concentration with glucose and pyruvate, respectively). While control by energy production was always low, it was doubled under glucose conditions compared with pyruvate (21.2% compared with 0.6% in glucose and 0.5% in pyruvate medium).

Table 3. Elasticity and flux control coefficients (control of overall contractile activity) under different conditions of activity.

<table>
<thead>
<tr>
<th>Ca(^{2+})</th>
<th>2.0 mM</th>
<th>Ca(^{2+})</th>
<th>3.5 mM</th>
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</thead>
<tbody>
<tr>
<td><strong>Pyruvate medium</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No. independent experiments</td>
<td>11</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Control coefficient, %§</td>
<td>−7.2±2.5‡</td>
<td>0.6±0.4</td>
<td>−12.9±5.5 † ‡</td>
</tr>
<tr>
<td>Elasticity coefficient</td>
<td>7.7±7.8 †</td>
<td>92.3±7.8 †</td>
<td>4.0±7.5 †</td>
</tr>
<tr>
<td><strong>Glucose medium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. independent experiments</td>
<td>5</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Control coefficient, %§</td>
<td>−2.9±1.2*†</td>
<td>0.8±0.5</td>
<td>−4.7±3.5 †</td>
</tr>
<tr>
<td>Elasticity coefficient</td>
<td>21.2±12.0†</td>
<td>78.8±12.0†</td>
<td>14.9±8.5</td>
</tr>
</tbody>
</table>

*P < 0.01 between glucose and pyruvate medium; †P < 0.05 between glucose and pyruvate medium; §Error calculated by Monte-Carlo simulations.

Fig. 4. MoCA of the effects of calcium concentration changes in the perfusion medium on heart bioenergetics under pyruvate and glucose conditions. Calcium effects on both PCr producer and consumer modules were calculated as described in EXPERIMENTAL PROCEDURES. Direct effect refers to the Integrated elasticity (total effect minus indirect effect), and global response refers to the response coefficient of each module on the increase in contraction induced by calcium increase. The size of the arrows is proportional to calcium effect, and the figures represent the effect expressed as % change from starting condition (2.0 mM Ca\(^{2+}\)). Pyruvate: (actual values ± error calculated from 500 Monte-Carlo simulations). Direct effect 41.4 ± 21.3 and 36.4 ± 1.8, global effect 3.2 ± 1.7 and 33.6 ± 1.7, and indirect effect −4.7 ± 0.2 and 0.4 ± 0.02. Glucose: direct effect 11.4 ± 3.0 and 21.5 ± 3.0, global effect 2.4 ± 0.6 and 17.0 ± 2.3, and indirect effect 8.0 ± 0.6 and −2.2 ± 0.2.
7.7% \( P < 0.05 \), at low calcium and 14.9% compared with 4.0% \( P < 0.05 \), at high calcium concentration). The higher control exerted by the producer under glucose conditions is the direct consequence of the lower elasticity of this module, compared with pyruvate conditions, due to the influence of glycolysis enzymes. All of these data show that energetic control of heart contraction is not distributed among the modules but resides almost entirely on the energy consumer and is not significantly changed during the increase in heart metabolic rate by \( \text{Ca}^{2+} \) (12). As these experiments were carried out at two different cytosolic \( \text{Ca}^{2+} \) concentrations, we may now apply the regulation analysis to get insights in the \( \text{Ca}^{2+} \) effect.

**Regulation Analysis**

Taking into account that \( \text{Ca}^{2+} \) may possibly directly activate both the producer and the consumer of \( \text{PCr}/\text{ATP} \), we need to discriminate these specific effects from indirect effects induced by internal changes in metabolites (i.e., \( \text{PCr} \)). All of the information is available from the MoCA carried out under two different \( \text{Ca}^{2+} \) conditions. These indirect effects may be deduced from the total measured effect induced by \( \text{Ca}^{2+} \) activation (RPP and \( \text{PCr} \) concentration changes) and calculated elasticities of each module toward \( \text{PCr} \) (see Experimental Procedures, results are presented in Fig. 4). Then direct effect of \( \text{Ca}^{2+} \) on each module (integrated elasticity) may be calculated as the total effect observed on contraction minus the indirect effects. As seen before, the paradigm of heart contraction is that only slight changes in \( \text{PCr} \) are associated with activity increase (30). Therefore, very small indirect effects of \( \text{Ca}^{2+} \) are involved. The results presented in Fig. 4 clearly show that a direct mitochondrial activation occurs in heart in response to calcium increase. With pyruvate as respiratory substrate, this direct mitochondrial activation by \( \text{Ca}^{2+} \) is equivalent to myofilament activation itself (Fig. 4A). However, with glucose as substrate, producer activation is significantly lower than myofilaments activation (Fig. 4B). These results may be explained by an absence of activation of glycolysis by \( \text{Ca}^{2+} \), since under these conditions glycolysis is part of the producer as pyruvate supplier to mitochondria.

The last information obtained from the regulation analysis is the global response to \( \text{Ca}^{2+} \) of heart contraction through each module. This global effect is calculated as the product of module activation by its control coefficient on total energy flux. As a consequence, the global effect on the flux is proportional to the direct effect on the module, and a strong effect on the flux requires both a direct effect and a significant control coefficient. Therefore, global effect quantifies how strongly the effector acts on the system through each route (10). Here, we get information on the overall effect of \( \text{Ca}^{2+} \) on heart contraction, which is almost entirely due to myofilaments activation under all conditions. This result is the direct consequence of the very low control exerted by the energy producer on contraction, due to the high sensitivity of mitochondria to \( \text{PCr} \) changes.

In conclusion, this study illustrates the potential use of the modular control and regulation analyses in combination with noninvasive techniques like \( ^{31} \text{P} \)-labeled NMR for the study of in situ energetics in beating heart. The low control by mitochondria on contraction is due to its high sensitivity to changes in energetic intermediates. In other words, these results show that heart contraction should not be sensitive to modulations of mitochondrial activity, but will be highly sensitive to any modulation of the contraction system. Energy flux in rat heart contraction is therefore mostly controlled by \( \text{Ca}^{2+} \) activation of myofilament ATPases. Regulation analysis not only demonstrated that a parallel activation of both energy producer and consumer occurs during calcium activation (direct effect), but also that the inotropic effect is the consequence of myofilament activation and not to the direct effect of calcium on mitochondria (global effect). The direct mitochondrial activation by \( \text{Ca}^{2+} \) is, however, crucial for the homeostasis of energetic intermediates and for heart physiologic response to \( \text{Ca}^{2+} \) increase (27).

MoCA is currently the only approach applicable to intact beating heart that gives information on the quantitative internal controls and regulations of integrated organ function. We show here that regulation analysis (8, 10) has the potential to provide a quantitative description of how an effector leads the system to move from a steady state to a new one. This approach should be fruitfully applied to the diagnosis of heart dysfunctions or drug effect. It will give two levels of information: the quantitative description of the effect on each module (direct effect) and the consequences of these modulations on heart contraction (global effect).

**REFERENCES**


