Am J Physiol Regul Integr Comp Physiol 295: R1891–R1897, 2008.
First published October 1, 2008; doi:10.1152/ajpregu.90548.2008.

Modular control analysis of effects of chronic hypoxia on mouse heart

Guillaume Calmettes,[1] Véronique Deschodt-Arsac,[1] Eric Thiaudière,[1] Bernard Muller,[2] and Philippe Diolez[1]

[1]*Résonance Magnétique des Systèmes Biologiques, UMR5536, Centre National de la Recherche Scientifique and* [2]*Laboratoire de Pharmacologie de l'UFR Pharmacie, Institut National de la Santé et de la Recherche Médicale U885, Université Victor Segalen Bordeaux 2, Bordeaux, France*

Submitted 30 June 2008; accepted in final form 26 September 2008

Calmettes G, Deschodt-Arsac V, Thiaudière E, Muller B, Diolez P. Modular control analysis of effects of chronic hypoxia on mouse heart. *Am J Physiol Regul Integr Comp Physiol* 295: R1891–R1897, 2008. First published October 1, 2008; doi:10.1152/ajpregu.90548.2008.—Modular control analysis (MoCA; Diolez P, Deschodt-Arsac V, Raffard G, Simon C, Santos PD, Thiaudiere E, Arsac L, Franconi JM. *Am J Physiol Regul Integr Comp Physiol* 293: R13–R19, 2007) was applied here on perfused hearts to describe the modifications of the regulation of heart energetics induced in mice exposed to 3-wk chronic hypoxia. MoCA combines ^{31}P-NMR spectroscopy and modular (top down) control analysis to describe the integrative regulation of energy metabolism in the intact beating heart, on the basis of two modules [ATP/phosphocreatine (PCr) production and ATP/PCr consumption] connected by the energetic intermediates. In contrast with previous results in rat heart, in which all control of contraction was on ATP demand, mouse heart energetics presented a shared control of contraction between ATP/PCr-producing and -consuming modules. In chronic hypoxic mice, the decrease in heart contractile activity and PCr-to-ATP ratio was surprisingly associated with an important and significant higher response of ATP/PCr production (elasticity) to PCr changes compared with control hearts (−10.4 vs. −2.46). By contrast, no changes were observed in ATP/PCr consumption since comparable elasticities were observed. Since elasticities determine the regulation of energetics of heart contraction, the present results show that this new parameter may be used to uncover the origin of the observed dysfunctions under chronic hypoxia conditions. Considering the decrease in mitochondrial content reported after exposure to chronic hypoxia, it appears that the improvement of ATP/PCr production response to ATP demand may be viewed as a positive adaptative mechanism. It now appears crucial to understand the very processes responsible for ATP/PCr producer elasticity toward the energetic intermediates, as well as their regulation.

perfused mouse heart; systems biology; ^{31}P-labeled magnetic resonance spectroscopy

CHRONIC HYPOXIA (CH) induces numerous adaptative changes in heart physiology, including remodeling. Indeed, in addition to the effect of lowered oxygen availability on oxidative cardiac metabolism, the heart is submitted to an increased workload as a result of the hypoxia-induced pulmonary hypertension (21, 22). This pathological situation induces complex structural, hormonal, and biochemical modifications that affect both energy-producing and energy-consuming processes in heart cells (see Refs. 20, 33). Concerning energy-producing modifications, an important reduction in the mitochondrial mass has been observed, associated with the reduction of the activities of respiratory chain complexes (29). In parallel, several studies reported a metabolic switch from fatty acid to carbohydrate utilization after CH (13, 25, 28, 36, 37). In regard to energy-consuming processes, one of the early mechanisms observed in isolated mammalian cardiomyocytes submitted to acute hypoxia is a downregulation of protein and RNA synthesis (9, 34). Moreover, biochemical investigations under CH also revealed a modification of the contractile apparatus itself, characterized by an increase in β-myosin heavy chain expression in both ventricles (35). These modifications of ATP/phosphocreatine (PCr) production and consumption processes are concomitant with the alteration of the phosphorylated metabolite content of the heart during CH, mainly a PCr decrease (23, 30, 31), indicative of heart energetics failure (19). Calcium ion (Ca^{2+}) plays a crucial role in excitation-contraction coupling, and in vitro studies (2–4) as well as our more recent in situ study (17) have evidenced that Ca^{2+} also directly activates mitochondrial oxidative phosphorylation. In CH, an alteration of calcium homeostasis associated with a decrease in calcium transients and resulting heart contractility has been reported (9, 34).

In the present study we applied our new modular control analysis (MoCA), an integrative approach to intact beating heart energetics (16, 17), to better understand the overall effect as well as the relative importance of these various modifications induced by CH on the regulation of energetics in hearts from mice submitted to hypobaric hypoxic conditions. Indeed, an appealing aspect of modular network analysis is the feature of "emergence," in which unexpected new properties arise from the cooperative interactions (integration) between biological processes (see Ref. 41 for review). The cardiovascular and muscular biochemical network has an intuitively modular structure (41); therefore, by associating noninvasive ^{31}P-magnetic resonance spectroscopy (MRS) with the analytic tools of metabolic control analysis (5, 8), we have developed an experimental integrative approach to in situ cardiac (16, 17) and skeletal (1) muscle energetics. In situ MoCA gives quantitative information on the internal control and regulation of integrated organ function and represents a powerful way to investigate pathological or drug effects. In MoCA, heart energetics is described as an ATP/PCr producer and ATP/PCr consumer system connected by the pool of the energetic intermediates [free energy of ATP hydrolysis (ΔGp), PCr, ATP, ADP, P_i]. A description of internal regulation and control of heart contraction under specific conditions is obtained after experimental determination of the kinetic properties (elasticity coefficients) of both supply (ATP/PCr producer) and demand (ATP/PCr consumer) modules toward the intermediates. In contraction

Address for reprint requests and other correspondence: P. Diolez, Résonance Magnétique des Systèmes Biologiques, UMR5536 CNRS-Université Victor Segalen Bordeaux 2, 146 rue Léo-Saignat, 33076 Bordeaux cedex, France (e-mail: diolez@rmsb.u-bordeaux2.fr).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

processes, the rapid shuttling of PCr allows the regeneration of ATP, maintaining low ADP concentration and maximizing ΔGp. Consequently, ATP (and ΔGp) may be considered as the local currency, while PCr is the major global intermediate (41). Determined in the intact beating heart, the elasticity coefficients toward the global intermediate PCr quantify the sensitivity of each module to the energetic intermediates and can therefore be used to detect any significant modification in the enzymatic processes of the module. MoCA has been used recently to demonstrate the parallel activation of both ATP/PCr producer and consumer by Ca^{2+} acting as an external effector (17) and during activation of perfused heart contraction by epinephrine (27). The application of MoCA on isolated, perfused hearts from mice subjected to a chronic hypoxic environment was carried out in order to highlight which of the above changes induced by CH really affect the very mechanisms of contractile function.

In the present study, MoCA analysis revealed that control of heart contraction energetics in mouse heart is clearly different from that in rat heart. Indeed, whereas control resides almost entirely in the energy consumer in rats, we observed a distribution among ATP/PCr-producing and ATP/PCr-consuming processes in mice. By comparison with control mice, MoCA analysis of CH mice evidenced an increase in the sensitivity of energy supply toward the intermediates after CH acclimatization, i.e., better response to ATP demand. This result is surprising if we consider the severe mitochondrial alteration already reported (29). By contrast, no change in energy demand sensitivity was noted in this study, indicating that the integrated consumer module response is hardly affected by CH. The consequence for the integrative cardiac energetics is an unexpected decrease in the control by energy supply, which may represent a compensation mechanism for the decrease in mitochondrial activity.

MATERIALS AND METHODS

Chronic hypoxia. Female Swiss mice (aged 6 wk, weighing 18–23 g) were separated into two groups. One group (CH mice) was exposed to a simulated altitude of 5,000 m (barometric pressure 380 mmHg) in a well-ventilated, temperature-controlled hypobaric chamber for 21 days. The chamber was opened briefly three times a week for cleaning of the cages and feeding of the animals. The other control group (normoxic mice, control) was maintained under ambient normoxic conditions [inspired O_2 fraction (FI_{O_2}) = 21%], with the same 12:12-h light-dark cycles. Free access to a standard mouse diet and water was allowed throughout the exposure period. The investigation conforms to the National and European Research Council Guide for the care and use of laboratory animals. P. Diolez has been attributed a permanent license to conduct experiments on animals by the Service Vétérinaire de la Santé et de la Protection Animale of the Ministère de l'Agriculture et de la Forêt (03/17/1999).

Heart perfusion. Animals were killed by cervical dislocation, and the heart was quickly excised and immediately submerged in ice-cold physiological solution. The aorta was cannulated, and hearts were perfused in a non-recirculating servo pump system (EMKA Technologies, Paris, France) at constant pressure (100 mmHg) with a Krebs-Henseleit buffer containing (in mmol/l) 108 NaCl, 5.9 KCl, 1.2 $MgSO_4$, 25 $NaHCO_3$, 0.5 EDTA, 1.1 mannitol, 11 glucose, 10 pyruvate, and 2.5 $CaCl_2$, pH 7.35. The perfusion was oxygenated with a 95% O_2-5% CO_2 mixture and a hollow-fiber membrane oxygenator (100 HG Fiber Dialyzer, GAMBRO).

A stab wound was made in the apex of the left ventricle to allow drainage of any fluid that might accumulate in the ventricular cavity (drain excess volume arising from Thebesian or arterioluminal sources). A fluid-filled latex balloon was inserted into the left ventricle via the left atrium for measurement of the left ventricular developed pressure and was connected to a P23 DB Gould-Statham pressure transducer through a fluid-filled polyethylene catheter.

Perfusion pressure, coronary flow, heart rate, and left ventricular developed pressure were measured continuously. Signals were recorded with dedicated software (IOX, EMKA Technologies). Mechanical performance of the heart was evaluated as the product of heart rate by developed pressure [rate-pressure product (RPP), in mmHg/min].

Each heart was placed inside a 10-mm NMR tube and transferred into a heated (37°C) 10-mm probe inside a 9.4-T superconducting magnet spectrometer (Bruker DPX 400-MHz Avance) for ^{31}P-NMR spectroscopy. All measurements were performed after a 20-min stabilization period.

NMR measurements. Pulsed Fourier-transformed ^{31}P-NMR spectra were obtained with a 9.4-T superconducting magnet (Bruker, Karlsruhe, Germany) with a 9-cm bore. The hearts were inserted into a ^{1}H/^{31}P double-tuned 10-mm probe. The probe was tuned to the phosphorus resonance frequency of 161.94 MHz, and the magnetic field homogeneity was optimized by shimming on the proton signal coming from the heart and the surrounding medium. Partially saturated ^{31}P-NMR spectra (14.5-μs radio frequency pulse, 60° flip angle, 2.18-s repetition time, 4,096 data points, reception bandwidth 5,196 Hz, 150 acquisitions, 10-Hz Lorentzian filter before Fourier transformation) were obtained without proton decoupling and were acquired within 4 min 59 s. Resonance areas were corrected after determination of T1 relaxation times of each phosphorus metabolite on perfused mouse hearts under the same experimental conditions.

Spectra were then analyzed with Igor Pro software (Wavemetrics, Lake Oswego, OR) as a sum of Lorentzian-Gaussian line-shaped resonances to determine the areas of the nucleoside triphosphate (ATP) and PCr resonances. The β-ATP resonance area was fixed at 11.8 mmol/l (18) for the first spectra and used for the internal calibration of PCr in the NMR spectra. The PCr-to-ATP ratio was calculated as the PCr-to-β-ATP resonance area ratio.

Modular control analysis. The principles of MoCA have been extensively described elsewhere (17), and only a short description will be found here. In MoCA, complex systems may be simplified by grouping reactions and reactants into large modules connected by a small number of explicit intermediates (6). We applied this to the study of energy transfer during contraction in heart by defining two modules (called producer and consumer) linked by energetic intermediates. In the working heart, because ATP concentration did not change under physiological conditions of heart activity, PCr was chosen as the representative intermediate between energy production and consumption. The producer module is then defined as all the steps from substrate and oxygen supply to ATP and PCr production (16, 17). The consumer module comprises all the ATP-consuming processes occurring during contraction (myosin ATPases and calcium reuptake by sarcoplasmic reticulum and cell membrane) (16, 17). Continuous noninvasive ^{31}P-NMR spectroscopy of perfused heart gives access to all energetic intermediates, including the representative intermediate, PCr. The energetic flux through the system was simultaneously assessed as the contractile activity measured by RPP (see above).

Following the principles of MoCA, control coefficients over the energy flux in heart contraction were calculated from the overall elasticities of the producer and consumer modules toward the intermediate (5). In MoCA, these elasticities ($\varepsilon_{PCr}^{Module}$) are calculated from the changes in flux (RPP) and PCr concentration ([PCr]) under steady-state conditions induced by a slight modulation of the activity of the other module (see Refs. 16, 17 for details). Experimentally, three different contraction steady states must be obtained in order to calculate elasticity coefficients, corresponding to

the reference state flanked by the two specific modulations of the modules (see Fig. 1). After heart stabilization, changes in [PCr] were induced by increasing internal balloon volume in order to trigger increased heart contraction (RPP) (4) and to calculate producer elasticity (ε^{P}_{PCr}). Because of the interdependence of the left and right ventricles (12) the volume of the left ventricle was reduced after CH, because of right ventricle hypertrophy. Therefore, balloon volume was increased from 7.5 μl to 15 μl in CH mice and from 10 μl to 20 μl in control mice. The steady states obtained after balloon pressure increase were deduced from the optima of the volume-pressure curves (results not shown) and considered as the reference state for the experimental determination of both elasticities. The second modulation concerned the producer module in order to determine consumer module elasticity (ε^{C}_{PCr}). For this purpose, a low cyanide concentration (0.3 mM NaCN) combined with 75 μM iodoacetic acid (IAA) was used to slightly inhibit mitochondrial cytochrome oxidase and totally (>95%) inhibit glycolysis at the level of glyceraldehyde-3-phosphate dehydrogenase (11), respectively. ε^{C}_{PCr} was calculated from the relative changes in contractile activity (RPP) and [PCr], in the ratio between the reference state and the new state of "low supply" reached.

The control coefficients of both modules (C^{Flux}_{P} and C^{Flux}_{C}) were then calculated from experimentally measured ε^{C}_{PCr} and ε^{P}_{PCr} according to summation and connectivity theorems (26). These experiments and subsequent calculations allow the complete description of the control pattern of the energetics of heart contraction under the conditions studied.

Statistical analysis. Experimental values are reported as means ± SD for the numbers of independent mouse hearts indicated. Statistical comparison between groups (hypobaric vs. control) was performed by one-way analysis of variance and post hoc Tukey honestly significant difference (HSD) tests. $P < 0.05$ and $P < 0.01$ stand for significant difference levels.

RESULTS

Effects of chronic hypoxia on morphometrics and cardiac contractile function. Heart weight was significantly increased after 3-wk exposure to CH (Table 1, $P < 0.01$). As already reported, body weight was significantly lower in CH mice (Table 1), attributable to the decrease in food intake observed during exposure to CH (14). Therefore, when expressed relative to body weight, total heart mass was increased by 33% at the end of CH exposure.

An example of the measurements obtained during MoCA-designed experiments is presented in Fig. 1. RPP and ^{31}P-MRS spectra were measured simultaneously during the specific modulations of the steady-state perfused heart contraction. Figure 1 shows the three successive steady states (see below and MATERIALS AND METHODS for details): inhibition of contraction (low balloon volume; *state a*), reference state (optimal balloon volume; *state b*), and inhibition of ATP/PCr production (*state c*). In this typical experiment, it can be seen that CH mice hearts were mainly characterized by a lower RPP. Table 2 describes the differences between control and CH hearts under reference-state conditions in regard to all of the measured parameters. While heart rate was not affected in CH, the left ventricular developed intraventricular pressure was significantly lower for CH (−50%) compared with control hearts (Table 2). The decrease in developed pressure induced a 56% significant decrease in heart work as reflected by RPP. The values of metabolite contents determined by NMR spectroscopy of the perfused hearts showed a significant 10% decrease in PCr-to-ATP ratio in CH hearts (Table 2, $P < 0.01$). These results may be related to the classical energetic failure observed in hypoxia (19, 32, 40).

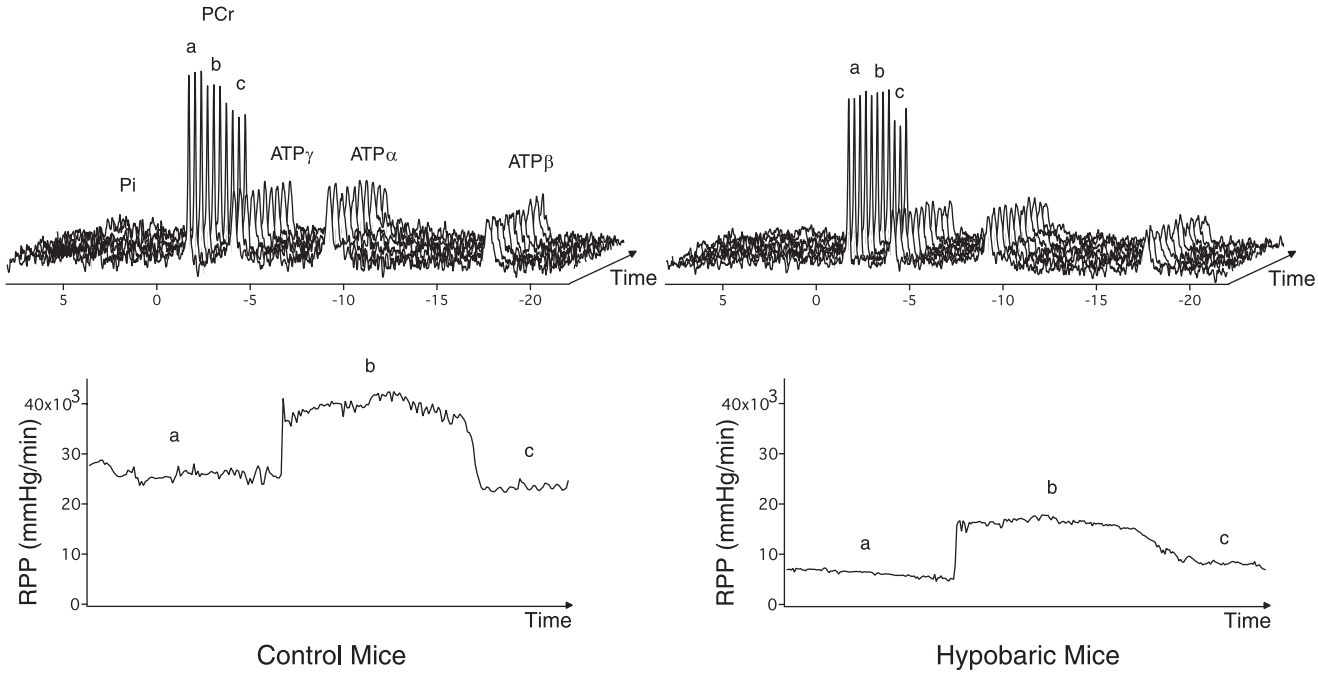

Fig. 1. Typical phosphocreatine (PCr) and rate-pressure product (RPP) records from control and hypobaric mice carried out to calculate producer and consumer elasticities in a single isolated, perfused heart. RPP and ^{31}P magnetic resonance spectroscopy (MRS) spectra were measured simultaneously during modulation of the steady state. MRS spectra were analyzed to assess steady values of energetic intermediates. The magnitude of the RPP was averaged over the corresponding period of time. *a*, Inhibition of contraction (low balloon volume); *b*, reference state (optimal balloon volume); *c*, inhibition of ATP/PCr production [NaCN + iodoacetic acid (IAA)].

Table 1. *Effect of hypobaric hypoxia on body and heart mass*

Morphometrics	Control Mice (n = 29)	Hypobaric Mice (n = 25)
Body weight, g	30.74±1.70	28.61±2.08*
Heart weight, mg	136±23	167±14†
Heart weight/body weight (×1,000)	4.42±0.75	5.87±0.48†

Values are means ± SE. Differences were tested by 1-way analysis of variance and post hoc Tukey honestly significant difference (HSD) tests. *P < 0.05, †P < 0.01 between control and hypobaric mice.

MoCA analysis. The first step in the analysis is the determination in intact heart of the kinetic interactions (elasticities) of the two modules (ATP/PCr producer and consumer) with [PCr] (17). With this aim, a specific modulation of one module is carried out in order to induce a change in [PCr] and measure the effect on the activity of the other module (RPP). To determine the producer response to PCr changes, myofilament Ca^{2+} sensitivity was enhanced dynamically by stretching the myofilaments slightly (Frank-Starling effect), through a balloon inserted into the ventricle. In this study, the experiment was started under low-pressure conditions, and the balloon was further inflated to reach the optimum contractile activity of the heart as the reference steady state (Fig. 1; see MATERIALS AND METHODS for details). This increase in balloon pressure induced a small decrease in [PCr] associated with an increase in heart contractile activity (RPP), reflecting the response of the producer module to the induced change in PCr (Fig. 1). As to the producer, since any mitochondrial inhibitor will do, cyanide (at very low concentration, 0.3 mM) was chosen for this study to determine consumer elasticity to PCr. An important difference from perfused rat heart (17) is the utilization of both glucose and pyruvate as substrates for perfused mouse heart (24); therefore the mild mitochondrial inhibition was combined with a severe inhibition of glycolysis by IAA. Inhibition of the ATP/PCr producer induced a decrease in PCr and a concomitant decrease in RPP (Fig. 1), in both CH and control mice. Table 3 presents RPP and PCr content values for the two groups under the reference steady state, as well as the relative changes observed during each specific modulation. These results represent the necessary set of data required for compared MoCA analysis of CH and control perfused hearts. The main differences were observed after increasing intraventricular pressure (consumer activation). CH hearts presented a much higher response of producer (+43.3% increase in RPP) compared with control hearts (+27.2%). Surprisingly, this higher increase in RPP was associated with a smaller decrease in [PCr] (−6% vs. −15%). By contrast, the producer inhibition by cyanide and IAA was responsible for about the same effect in both CH and control hearts for PCr as well as RPP (−25% change in [PCr] for a −40% decrease in energy flux) (Table 3).

Table 2. *Effect of hypobaric hypoxia on cardiac contractile function and metabolite content*

	Control Mice (n = 29)	Hypobaric Mice (n = 25)
LV cardiac parameters		
Heart rate, beats/min	436±47	416±56
Developed pressure, mmHg	78±36	39±15†
RPP, mmHg/min	34,382±19,008	15,175±6,709†
Metabolite content		
[PCr], mM	13.62±2.82	11.69±1.88*
[ATP], mM	11.42±1.34	10.93±0.66
[PCr]/[ATP]	1.19±0.16	1.07±0.14*

Values are means ± SE. Heart rate and left ventricle (LV) developed pressure were measured with a fluid-filled balloon inserted into the LV. Metabolic contents of the myocardium from hypoxic and control mice were measured by ^{31}P-NMR. [PCr], [ATP], phosphocreatine and ATP concentrations; RPP, rate-pressure product. Differences were tested by 1-way analysis of variance and post hoc Tukey HSD tests. *P < 0.05, †P < 0.01 between control and hypobaric mice.

Table 3. *Relative changes in PCr and in contractile activity (RPP) from reference steady state induced by small change in intraventricular pressure or by mild mitochondrial inhibition by cyanide*

	Control Mice	Hypobaric Mice
Reference steady state		
[PCr], mM	13.62±2.82	11.69±1.88*
RPP, mmHg/min	34,382±19,008	15,175±6,709†
Change in intraventricular pressure		
PCr (Δ[PCr]/[PCr]$_i$)	−14.7±6.1	−5.9±2.1†
RPP (ΔRPP/RPP$_i$)	+27.2±8.9	+43.3±12.2†
Cyanide + IAA inhibition		
PCr (Δ[PCr]/[PCr]$_i$)	−28.2±14.4	−23.7±7.0
RPP (ΔRPP/RPP$_i$)	−40.4±8.8	−44.4±12.1

Values are means ± SE. RPP$_i$, initial RPP; [PCR]$_i$, initial [PCr]; IAA, iodoacetic acid. Differences were tested by 1-way analysis of variance and post hoc Tukey HSD tests. *P < 0.05, †P < 0.01 between control and hypobaric mice.

The above relative changes in RPP and [PCr] were used to calculate the elasticity coefficients of the producer and consumer modules reported in Fig. 2 as well as the contraction control coefficients presented in Table 4, which represent the control exerted by each module (producer and consumer) on the overall contractile activity. In control hearts, the absolute

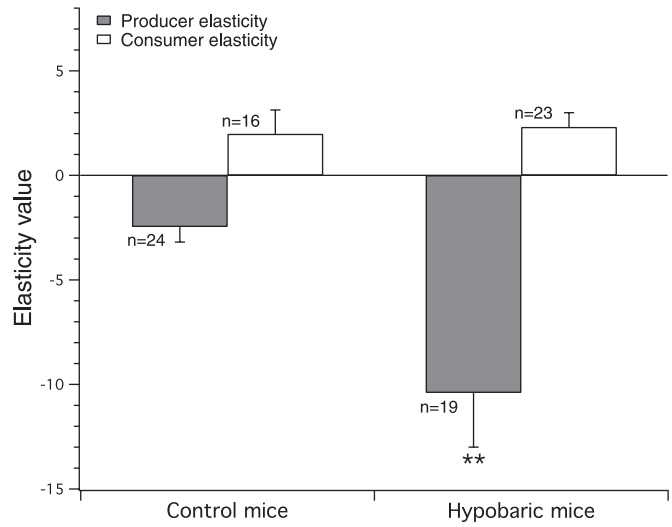

Fig. 2. Elasticity of producer and consumer modules in control and hypoxic hearts. Filled bars, producer elasticity for control (−2.46 ± 0.73) and hypobaric (−10.40 ± 2.60) mice; open bars, consumer elasticity for control (1.98 ± 1.15) and hypobaric (2.31 ± 0.69) mice. Data are presented as means ± SE. Differences were tested by 1-way analysis of variance and post hoc Tukey honestly significant difference tests. **P < 0.01 between control and hypobaric mice.

Table 4. *Control coefficients of producer and consumer modules in control and hypoxic hearts*

	Control Coefficient Over Flux, %	
	Control Mice (n = 12)	Hypobaric Mice (n = 21)
Producer	46±16	18±7†‡
Consumer	54±16	82±7†‡

Values are means ± SE. Differences were tested by 1-way analysis of variance and post hoc Tukey HSD tests. †$P < 0.01$ between control and hypobaric mice; ‡$P < 0.01$ between producer and consumer.

values of both elasticity coefficients were almost identical between producer and consumer (−2.46 ± 0.73 and 1.98 ± 1.15, respectively). The direct consequence is that control of heart contraction is almost perfectly shared between producer and consumer (Table 4) in control perfused mouse heart. This conclusion is a striking difference with our previous results on perfused rat heart, in which control is almost entirely in the consumer in the presence of pyruvate or glucose as substrate (16, 17).

By comparison, we observed an important increase in producer elasticity to changes in PCr with CH hearts (−10.40 ± 2.60 for CH compared with −2.46 ± 0.73 for control; $P <$ 0.01) but no significant change in consumer elasticity (2.31 ± 0.69 and 1.98 ± 1.15 for CH and control, respectively). The distribution of control of contraction in CH hearts calculated from these elasticities was not balanced and was about 20% for the producer and 80% for the consumer (Table 4). These results demonstrate that one important consequence of CH on heart function is a marked decrease in the control exerted by ATP/PCr producer on contraction energetics, which is due to a surprising increase in the producer's response (elasticity) to ATP demand by contracting myofibrils.

DISCUSSION

The kinetic interaction of ATP/PCr-producing and -consuming modules with PCr takes a central place in cardiac energetics (healthy and pathological) since it characterizes the transfer of information from ATP/PCr consumer (increase in contractility induced by Ca^{2+} transients) to ATP/PCr producer via the modification of [PCr]. Consequently, steady-state contraction characteristics strongly rely on these interactions, which are the elasticities of the different modules toward PCr, experimentally determined by MoCA in the present study.

The results obtained with CH mice presented here show a marked perturbation of heart contractile activity and especially in the steady-state concentrations of the energetic intermediates, since an important decrease in PCr-to-ATP ratio was observed in CH mice associated with a decrease in overall contractile activity by 56%. Indeed, the modification in phosphorus metabolite content is an index of cardiac diseases (19) and has been described in detail in chronic hypoxic hearts of several animal models (23, 30, 31). From our previous studies, the changes in the intermediates cannot be accounted for by the decrease in contractile activity, but uncover fundamental modifications in the mechanisms responsible for steady-state contraction energetics. This view is supported by previous findings that exposure of rats to CH triggers an alteration in mitochondrial respiratory chain complexes (29) and reduces both release and reuptake of Ca^{2+} by sarcoplasmic reticulum and therefore Ca^{2+} transients (34, 39). Such an alteration in contractility has already been observed in other cardiac disease models, e.g., with spontaneously hypertensive rats (15). Together, all these data indicate the occurrence in CH hearts of a decrease in oxidative phosphorylation by two mechanisms: a decrease in mitochondrial activity (29) worsened by a lack of activation by a decrease in Ca^{2+} transients (17), which also decreases contractile activity directly (34, 39).

However, we also observed in the present study a surprising improvement of the contractile response to the increase in balloon pressure (Frank-Starling effect) after the 3-wk period of acclimation to CH. Indeed, in response to an even smaller increase in ventricular pressure (balloon volume), CH hearts displayed a significantly bigger response in RPP compared with control hearts, associated with a much smaller decrease in PCr (Fig. 1). Since careful experimentation on skinned cardiomyocytes has demonstrated the absence of modification of the Frank-Starling response after CH (10), it appeared difficult to explain this improved response to higher ATP demand by contraction, especially if we consider the above results showing a decrease in mitochondrial activity and activation in CH hearts.

Because of the complex interactions occurring in the heart and the apparent contradictory data presented here, an integrative approach appeared necessary in order to gain a comprehensive description of the very mechanisms responsible for the changes in contractility observed with CH hearts. In this study, we applied MoCA to study the changes in the elasticities of the ATP/PCr-producing and -consuming modules toward PCr in CH hearts. The main interesting feature when applied to the study of pathologies is the consideration that only the module presenting an overall modified elasticity by the pathology has significant impact on intact heart function.

First, the present study on the control mouse perfused heart showed equivalent elasticities of producer and consumer modules, and consequently an almost shared repartition of the control of contraction. This result was surprising in view of our previous results in rat heart, in which we showed that control was almost totally in the consumer (ATP demand, between 90% and 95%), because of a much higher elasticity of the producer toward PCr (17). While this important difference between mouse and rat hearts needs further investigation, a possible explanation could reside in the very high contractile activity of the mouse heart. Indeed, comparable RPPs were recorded with mouse and rat hearts under our conditions (~30,000–40,000 mmHg/min), while mouse heart is ~10 times smaller and has a much higher rate (400 compared with 250 beats/min for rat heart). We may therefore suggest that the elasticity of the ATP/PCr producer toward [PCr] could decrease at high cardiac contractile activity.

When applied to mice exposed for 3 wk to CH, MoCA revealed a surprising significant fourfold increase in the elasticity of the producer module to changes in PCr compared with control mice. In contrast, no modification in the elasticity of the ATP/PCr consumer was evidenced in this study. As discussed above, these results clearly show that the changes in the ATP/PCr producer module experimentally evidenced here are fully responsible for the observed changes in heart contraction energetics in CH perfused hearts. The most obvious process whose modification may be responsible for the change in the kinetic interaction between ATP/PCr producer and PCr is the adenylate translocator (ANT)-mitochondrial creatine kinase-

porin complex (7), which is at the interface between mitochondria and the energetic intermediates, including creatine/PCr. It has been already shown that binding of creatine kinase with mitochondrial membranes can be modulated by different factors, particularly calcium (38). The surprising increase in the elasticity of the ATP/PCr producer observed here, despite a decrease in global activity, emphasizes a new property of the system and could be considered as an adaptative mechanism developed in the heart under CH counteracting the decrease in activity and activation, both linked to the decrease in calcium transients. Strongly suggested from the present results, the role of Ca^{2+} and PCr concentrations in the modulation of ATP/PCr producer elasticity in healthy and CH hearts, and therefore in the overall regulation of cardiac energetics, needs to be clarified.

It appears crucial to further investigate this fundamental property, which is a direct consequence of organ integration and can only be assessed through integrative approaches applied to intact organs. MoCA appears to be a very promising approach since the noninvasive use of ^{31}P-NMR allows the possible use in vivo.

Perspectives and Significance

The present work is the first application of our modular control analysis to heart pathology. MoCA is one of the new systems biology approaches currently being developed and allows the integrative and noninvasive study of the energetics of intact beating heart. The results presented in this paper clearly show that integrative approaches effectively bring a new type of information providing new insights into the complex internal regulations of integrated organ function. In the near future systems biology approaches may be crucial in the actual attempts to link molecular events to physiology and pathology.

GRANTS

This work was supported by Centre National de la Recherche Scientifique (CNRS), Université Victor Segalen (Bordeaux 2), Conseil Régional de la Région Aquitaine, and Institut Fédératif de Recherche (IFR)4, Bordeaux, France.

REFERENCES

1. **Arsac LM, Beuste C, Miraux S, Deschodt-Arsac V, Thiaudiere E, Franconi JM, Diolez PH.** In vivo modular control analysis of energy metabolism in contracting skeletal muscle. *Biochem J* 414: 391–397, 2008.
2. **Balaban RS.** Cardiac energy metabolism homeostasis: role of cytosolic calcium. *J Mol Cell Cardiol* 34: 1259–1271, 2002.
3. **Balaban RS, Bose S, French SA, Territo PR.** Role of calcium in metabolic signaling between cardiac sarcoplasmic reticulum and mitochondria in vitro. *Am J Physiol Cell Physiol* 284: C285–C293, 2003.
4. **Bers DM.** Cardiac excitation-contraction coupling. *Nature* 415: 198–205, 2002.
5. **Brand MD.** Top down metabolic control analysis. *J Theor Biol* 182: 351–360, 1996.
6. **Brand MD, Curtis RK.** Simplifying metabolic complexity. *Biochem Soc Trans* 30: 25–30, 2002.
7. **Brdiczka D, Beutner G, Ruck A, Dolder M, Wallimann T.** The molecular structure of mitochondrial contact sites. Their role in regulation of energy metabolism and permeability transition. *Biofactors* 8: 235–242, 1998.
8. **Brown GC, Hafner RP, Brand MD.** A "top-down" approach to the determination of control coefficients in metabolic control theory. *Eur J Biochem* 188: 321–325, 1990.
9. **Casey TM, Pakay JL, Guppy M, Arthur PG.** Hypoxia causes down-regulation of protein and RNA synthesis in noncontracting mammalian cardiomyocytes. *Circ Res* 90: 777–783, 2002.
10. **Cazorla O, Ait Mou Y, Goret L, Vassort G, Dauzat M, Lacampagne A, Tanguy S, Obert P.** Effects of high-altitude exercise training on contractile function of rat skinned cardiomyocyte. *Cardiovasc Res* 71: 652–660, 2006.
11. **Chatham J, Gilbert HF, Radda GK.** Inhibition of glucose phosphorylation by fatty acids in the perfused rat heart. *FEBS Lett* 238: 445–449, 1988.
12. **Clyne CA, Alpert JS, Benotti JR.** Interdependence of the left and right ventricles in health and disease. *Am Heart J* 117: 1366–1373, 1989.
13. **Daneshrad Z, Garcia-Riera MP, Verdys M, Rossi A.** Differential responses to chronic hypoxia and dietary restriction of aerobic capacity and enzyme levels in the rat myocardium. *Mol Cell Biochem* 210: 159–166, 2000.
14. **Daneshrad Z, Novel-Chate V, Birot O, Serrurier B, Sanchez H, Bigard AX, Rossi A.** Diet restriction plays an important role in the alterations of heart mitochondrial function following exposure of young rats to chronic hypoxia. *Pflügers Arch* 442: 12–18, 2001.
15. **Delbridge LM, Connell PJ, Morgan TO, Harris PJ.** Contractile function of cardiomyocytes from the spontaneously hypertensive rat. *J Mol Cell Cardiol* 28: 723–733, 1996.
16. **Diolez P, Raffard G, Simon C, Leducq N, Dos SP, Canioni P.** Mitochondria do not control heart bioenergetics. *Mol Biol Rep* 29: 193–196, 2002.
17. **Diolez P, Deschodt-Arsac V, Raffard G, Simon C, Santos PD, Thiaudiere E, Arsac L, Franconi JM.** 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.
18. **Dos Santos P, Aliev MK, Diolez P, Duclos F, Besse P, Bonoron-Adele S, Sikk P, Canioni P, Saks VA.** Metabolic control of contractile performance in isolated perfused rat heart. Analysis of experimental data by reaction:diffusion mathematical model. *J Mol Cell Cardiol* 32: 1703–1734, 2000.
19. **Epstein FH.** MR in mouse models of cardiac disease. *NMR Biomed* 20: 238–255, 2007.
20. **Essop MF.** Cardiac metabolic adaptations in response to chronic hypoxia. *J Physiol* 584: 715–726, 2007.
21. **Falk JA, Kadiev S, Criner GJ, Scharf SM, Minai OA, Diaz P.** Cardiac disease in chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 5: 543–548, 2008.
22. **Han MK, McLaughlin VV, Criner GJ, Martinez FJ.** Pulmonary diseases and the heart. *Circulation* 116: 2992–3005, 2007.
23. **Hochachka PW, Clark CM, Holden JE, Stanley C, Ugurbil K, Menon RS.** ^{31}P magnetic resonance spectroscopy of the Sherpa heart: a phosphocreatine/adenosine triphosphate signature of metabolic defense against hypobaric hypoxia. *Proc Natl Acad Sci USA* 93: 1215–1220, 1996.
24. **Hoerter J, Gonzalez-Barroso MD, Couplan E, Mateo P, Gelly C, Cassard-Doulcier AM, Diolez P, Bouillaud F.** Mitochondrial uncoupling protein 1 expressed in the heart of transgenic mice protects against ischemic-reperfusion damage. *Circulation* 110: 528–533, 2004.
25. **Holden JE, Stone CK, Clark CM, Brown WD, Nickles RJ, Stanley C, Hochachka PW.** Enhanced cardiac metabolism of plasma glucose in high-altitude natives: adaptation against chronic hypoxia. *J Appl Physiol* 79: 222–228, 1995.
26. **Kacser H, Burns JA.** The control of flux. *Symp Soc Exp Biol* 27: 65–104, 1973.
27. **Korzeniewski B, Deschodt-Arsac V, Calmettes G, Franconi JM, Diolez PH.** Physiological heart activation by adrenaline involves parallel activation of ATP usage and supply. *Biochem J* 413: 343–347, 2008.
28. **Leverve X.** Metabolic and nutritional consequences of chronic hypoxia. *Clin Nutr* 17: 241–251, 1998.
29. **Nouette-Gaulain K, Malgat M, Rocher C, Savineau JP, Marthan R, Mazat JP, Sztark F.** Time course of differential mitochondrial energy metabolism adaptation to chronic hypoxia in right and left ventricles. *Cardiovasc Res* 66: 132–140, 2005.
30. **Novel-Chate V, Aussedat J, Saks VA, Rossi A.** Adaptation to chronic hypoxia alters cardiac metabolic response to beta stimulation: novel face

of phosphocreatine overshoot phenomenon. *J Mol Cell Cardiol* 27: 1679–1687, 1995.
31. **Novel-Chate V, Mateo P, Saks VA, Hoerter JA, Rossi A.** Chronic exposure of rats to hypoxic environment alters the mechanism of energy transfer in myocardium. *J Mol Cell Cardiol* 30: 1295–1303, 1998.
32. **Omerovic E, Basetti M, Bollano E, Bohlooly YM, Tornell J, Isgaard J, Hjalmarson A, Soussi B, Waagstein F.** In vivo metabolic imaging of cardiac bioenergetics in transgenic mice. *Biochem Biophys Res Commun* 271: 222–228, 2000.
33. **Ostadal B, Kolar F.** Cardiac adaptation to chronic high-altitude hypoxia: beneficial and adverse effects. *Respir Physiol Neurobiol* 158: 224–236, 2007.
34. **Pei JM, Kravtsov GM, Wu S, Das R, Fung ML, Wong TM.** Calcium homeostasis in rat cardiomyocytes during chronic hypoxia: a time course study. *Am J Physiol Cell Physiol* 285: C1420–C1428, 2003.
35. **Pissarek M, Bigard X, Mateo P, Guezennec CY, Hoerter JA.** Adaptation of cardiac myosin and creatine kinase to chronic hypoxia: role of anorexia and hypertension. *Am J Physiol Heart Circ Physiol* 272: H1690–H1695, 1997.
36. **Razeghi P, Young ME, Abbasi S, Taegtmeyer H.** Hypoxia in vivo decreases peroxisome proliferator-activated receptor alpha-regulated gene expression in rat heart. *Biochem Biophys Res Commun* 287: 5–10, 2001.
37. **Rumsey WL, Abbott B, Bertelsen D, Mallamaci M, Hagan K, Nelson D, Erecinska M.** Adaptation to hypoxia alters energy metabolism in rat heart. *Am J Physiol Heart Circ Physiol* 276: H71–H80, 1999.
38. **Schlattner U, Dolder M, Wallimann T, Tokarska-Schlattner M.** Mitochondrial creatine kinase and mitochondrial outer membrane porin show a direct interaction that is modulated by calcium. *J Biol Chem* 276: 48027–48030, 2001.
39. **Silverman HS, Wei S, Haigney MC, Ocampo CJ, Stern MD.** Myocyte adaptation to chronic hypoxia and development of tolerance to subsequent acute severe hypoxia. *Circ Res* 80: 699–707, 1997.
40. **Ten Hove M, Neubauer S.** MR spectroscopy in heart failure—clinical and experimental findings. *Heart Fail Rev* 12: 48–57, 2007.
41. **Weiss JN, Yang L, Qu Z.** Systems biology approaches to metabolic and cardiovascular disorders: network perspectives of cardiovascular metabolism. *J Lipid Res* 47: 2355–2366, 2006.