CK inhibition accelerates transcytotic energy signaling during rapid workload steps in isolated rabbit hearts

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The creatine kinase (CK)-catalyzed transfer of high-energy phosphate between ATP and phosphocreatine (PCr) is of central importance in muscle bioenergetics. The role played by CK in the regulation of myocyte energy transfer and signaling remains controversial (see for review Refs. 20, 33, 34). From the accepted temporal ATP/ADP buffering function of the CK/PCr system a progressively more complex model of energy transfer has evolved. The hypothesis of the "PCr shuttle" (3, 11, 33) providing energy transport or "spatial buffering" via PCr/creatine (Cr) and maintenance of optimal ATP-to-ADP ratios also incorporates compartmentation of CK isozymes in mitochondria (8, 18) and myofibrils (13, 32). Moreover, CK has been hypothesized to act as a metabolic control system in the regulation of cellular respiration (20). In opposition to this energy transfer theory remains the concept that the CK reaction operates in equilibrium as a "metabolic capacitor" between mitochondria and myofibrils allowing free diffusion of ATP and ADP, facilitated by PCr and Cr, to act as the energy transfer mechanism (15, 22, 23).

Recent studies in CK-blocked rat hearts (9, 10, 25) and CK knockout mice (29–31) show that normal workloads and high-energy phosphate levels can be maintained without active CK. However, increased performance in both isolated hearts and skeletal muscle is restricted. The loss of contractile reserve is accompanied by decreased free energy of ATP hydrolysis (ΔGATP), which is critical for maintaining the Ca2+-handling capacity of the sarcoplasmic reticulum (25). Two recent isolated heart studies suggest that the predominant effect of CK inactivity is increased ADP, leading to diastolic dysfunction and loss of myofibrillar compliance (12, 24). In human cardiac failure loss of CK activity, changes in CK isozyme distribution, and altered high-energy phosphate metabolite levels coexist (16, 19).

We previously developed a method to assess the delay time (tmito) in mitochondrial ATP synthesis after rapid increases in ATP hydrolysis induced by heart rate steps (28). 31P NMR experiments showed that PCr and P, change markedly faster than oxidative phosphorylation (7), and we suggested that changes in phosphate metabolite concentrations take place in or near myofibrils/ion pumps before they reach the mitochondria with some delay (27). Thus tmito reflects the transcytotic signaling speeds between myofibrils/ion pumps and mitochondria at submaximal levels of O2 consumption (7, 27, 28).

The aim of this study was to examine the effect of graded, irreversible CK inhibition [using iodoacetamide (IA) infusion] on the energy transduction speeds and contractile function of isolated rabbit hearts during paced workload steps. On the basis of the PCr shuttle operating as the optimal energy transfer system, it is predicted that blocking CK leads to longer response times (5, 18, 20). Indeed, prolonged response times found in the stunned rabbit heart were hypothesized to be caused by inhibition of CK (36). Our present results during graded inhibition of CK instead show an apparent quickening of ATP hydrolysis to synthesis signal coupling, suggesting that the cytosolic energy transfer function of CK is nonessential and can be bypassed by metabolite diffusion.

METHODS

Isolated heart preparation. All experiments were approved by the local animal ethics and experimentation authority...
The buffer was maintained at 37°C and continuously gassed with 5% CO₂ to obtain maximal vasodilation in our preparation.

The coronary perfusion pressure (CPP), systolic/diastolic ventricular pressure, and O₂ consumption ([\text{VO}_2]) were made at time point 1, 3, 4, 5, and 7. Calculations of response time of mitochondrial O₂ consumption (\text{t}_{\text{mito}}) were made during periods 2 and 6. Biochemical analysis of heart tissue followed experimental completion at time point 7. Iodoacetamide (IA) was infused between time points 3 and 4. HR, heart rate steps; ACS, arterial concentration step; EBS, intravascular indicator (Evans blue) step. For further description see METHODS.

Fig. 1. Schematic representing experimental protocol used in present study. Functional measurements [coronary perfusion pressure (CPP), systolic/diastolic ventricular pressure, and \text{O}_2 consumption] were made at time points 1, 3, 4, 5, and 7. Calculations of response time of mitochondrial \text{O}_2 consumption (\text{t}_{\text{mito}}) were performed (as outlined in Calculation of \text{t}_{\text{mito}}). Incorporating randomized heart rate steps from basal heart rate (135 beats/min) to 160, 190, and 220 beats/min and back plus the ACS and EBS steps at 135 and 220 beats/min. Hearts were then randomly assigned to one of four treatment groups (n = 7/group) to receive 0 (control), 0.1, 0.2, or 0.4 mM IA over the next 15 min. Concentrations given are final millimolar values in the perfusing solution after infusion of stock solutions (IA dissolved in H_2O) into a side arm of the aortic cannula at 1.2 ml/min with an infusion pump (Vickers Medical). Control hearts received only vehicle infusion. Time points 3–5 represent hemodynamic measurements made before and after IA infusion. Hearts were allowed 15 min for IA washout and reequilibration before the second series of
activities are reported as international units (1 IU) according to methods described by Bergmeyer (1). All enzyme production at 25°C and an absorbance wavelength of 340 nm before measurement of total CK activity coupled to NADH with bovine serum albumin as standard. Triton X-100 was removed for protein measurement by a modified Lowry method as described by Peterson (17) and is reported as milligrams of protein per milligram of dry heart tissue weight.

Biochemical assays. Tissue samples (5-10 mg) were cut from the freeze-dried heart sections and homogenized at 4°C for 20 s in 0.1 M potassium phosphate buffer containing 1 mM EDTA and 1 mM β-mercaptoethanol, pH 7.4. Aliquots were removed for protein measurement by a modified Lowry method as described by Peterson (17) and is reported as milligrams of protein per milligram of dry heart tissue weight with bovine serum albumin as standard. Triton X-100 was added to the homogenate at a final concentration of 0.1% before measurement of total CK activity coupled to NADPH production at 25°C and an absorbance wavelength of 340 nm (1). Adenylate kinase (AK) and glyceroldehyde-3-phosphate dehydrogenase (GAPDH) activities were also assayed at 25°C according to methods described by Bergmeyer (1). All enzyme activities were reported as international units (1 IU = µmol/min) per milligram of dry heart tissue weight.

Mitochondrial function. Mitochondria were isolated at the completion of experiments in a subset of control (n = 3) and 0.4 mM IA-treated (n = 3) hearts as described by Mela and Selzt (14). Briefly, 3–5 g of apical left ventricular tissue were excised into 4°C isolation solution containing (in mM) 225 mannitol, 75 sucrose, 1 EDTA, and 10 MOPS with 5 g/l albumin (pH = 7.25) and manually cut into small pieces. After the isolation solution was refreshed, 0.4 g protease was added and the tissue was homogenized and centrifuged at 850 g for 5 min. The supernatant was further centrifuged at 6,950 g for 10 min, and the remaining pellet was washed and spun twice more. The final pellet was resuspended in the isolation solution containing 4°C. Glutamate (5 mM) and malate (5 mM) were used as mitochondrial substrates, and rates of O₂ consumption were measured using a Clark-type O₂ electrode after respiration was stimulated with either 1 mM ADP or a combination of 20 mM Cr and ATP that was varied from 0.5–1.5 mM. CK activity in the mitochondrial fraction was assayed using the same method as for total tissue homogenate CK (1) and is expressed as international units per milligram of mitochondrial protein.

All chemical reagents used were of analytical grade and were obtained from Sigma Chemical (St. Louis, MO) or Boehringer Mannheim (Mannheim, Germany).

Statistical analysis. All data are presented as means ± SE. Comparisons among treatment groups were made using ANOVA with the dose of IA used as a grouping factor. The Newman-Keuls post hoc test was used to examine specific differences between group means. Two-way ANOVA (IA dose and heart rate) was used to analyze changes in tₐᵣ after or before treatment. Measurements made before and after treatment within groups were compared using the Student’s paired t-test or ANOVA for repeated measures as necessary. A value of P < 0.05 was considered statistically significant for all comparisons.

RESULTS

IA and hemodynamic function. Baseline contractile function assessed after equilibration (time point 1, Fig. 1) generated values of 93 ± 7 mmHg for systolic left ventricular pressure (SLVP), 4 ± 1 mmHg for end-diastolic pressure (EDP), 85 ± 2 mmHg for CPP, 14 ± 1 ml·min⁻¹·g wet wt⁻¹ for coronary flow, and 22 ± 1 µmol·min⁻¹·g dry wt⁻¹ for MVO₂. No statistically significant difference was observed between treatment groups in any of these parameters or in the wet (7.46 ± 0.39 g) or dry (1.25 ± 0.06 g) weight of hearts. The effect of IA infusion on SLVP, EDP, CPP, and MVO₂ is shown in Fig. 2 as percent changes between time points 3 and 4. There was a small decrease in SLVP in all groups (significant in 0.2 and 0.4 mM IA) and an increase in CPP to a similar degree. The significant rise in EDP that occurred in IA-treated hearts, an increase of 53 ± 9%, was high in relative terms because of the low baseline value of EDP but modest in absolute terms at 2.9 ± 0.4 mmHg in the 0.4 mM IA group. This effect of CK inhibition on EDP is consistent with previous rat heart studies using IA infusion and has been ascribed to increased ADP levels (24). MVO₂, like SLVP, decreased slightly in all groups after treatment, with the decrease in the 0.4 mM IA-treated hearts (9.8 ± 1.4%) reaching significance versus controls (4.6 ± 1.1%, P < 0.05). During the 15-min wash-out period contractile parameters did not change further (assessed at time point 5); rather, values were stable at their new levels before the second round of tₐᵣ calculations (period 6).

Contractile reserve, defined as the increase in RPP during a step in heart rate from 135 to 220 beats/min, measured before and after treatment was reduced by IA. This increase in RPP, 5,749 ± 524 mmHg/min (or 48 ± 5%), was not different among groups before treatment. It did decline over time in control hearts to 90 ± 2.8% of pretreatment values. This decreased to 88 ± 2.5, 79 ± 2.6 (P < 0.05 vs. control), and 80 ± 2.5 (P < 0.05 vs. control) % in the 0.1 mM, 0.2 mM, and 0.4 mM IA-treated hearts, respectively. The results show
that the ability of CK-blocked hearts to tolerate rapid workload increases is compromised.

Biochemical analysis of IA-treated hearts. The activity of CK, AK, and GAPDH enzymes and the protein content in tissue homogenates from the four heart groups appear in Table 1. Total CK activity in control hearts was reduced in a dose-dependent manner by IA to 14, 6, and 3% in 0.1, 0.2, and 0.4 mM IA-treated hearts, respectively. GAPDH, a sulfhydryl group containing glycolytic enzyme, was previously shown to be markedly inhibited by IA (9, 24). Its activity was indeed decreased by 23, 42, and 68% respectively, in the three treated groups. The AK system may also transfer high-energy phosphoryls in the myocyte (5, 6), and its activity in CK-blocked hearts was therefore examined. AK activity was 55-fold lower than CK activity in control hearts, and this was unchanged by IA treatment at any dose.

To confirm that oxidative phosphorylation was not inhibited in IA-treated hearts, we isolated mitochondria from three control and three 0.4 mM IA-treated hearts. Mitochondrial CK inhibition was verified by direct assay of mitochondrial CK and the lack of change was progressively reversed by increasing IA concentrations, with tmito increasing by 16 ± 6% in 0.1 mM IA-treated hearts (P < 0.05) but decreasing significantly by 20 ± 6 (P < 0.05) and 46 ± 6 (P < 0.01)% after treatment with 0.2 and 0.4 mM IA, respectively. The changes in these latter two groups were significant compared with changes in the control hearts (P < 0.05) and were independent of the heart rate step used (2-way ANOVA, P > 0.05). These results suggest that CK blockade with IA causes an apparent quickening in the metabolic coupling between oxidative phosphorylation and energy utilization.

Because transport and diffusion delay is subtracted during the tmito calculations, transport must be carefully assessed. The transport at 4.6 ± 0.4 s was not different between groups before treatment. It increased by 18 ± 5, 11 ± 5, 20 ± 5, and 34 ± 6% in control, 0.1 mM IA, 0.2 mM IA, and 0.4 mM IA groups, respectively (P < 0.05 vs. pretreatment). The increase in 0.4 mM IA-treated IA-treated hearts.

Table 1. Biochemical analysis of control and iodoacetamide-treated hearts

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>IA 0.1 mM</th>
<th>IA 0.2 mM</th>
<th>IA 0.4 mM</th>
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<tr>
<td><strong>Left ventricular homogenates</strong></td>
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<tr>
<td>CK activity, IU/mg dry heart wt</td>
<td>7.75 ± 0.53</td>
<td>11.1 ± 0.10*</td>
<td>0.44 ± 0.08*</td>
<td>0.25 ± 0.08*</td>
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<td>GAPDH activity, IU/mg dry heart wt</td>
<td>0.39 ± 0.01</td>
<td>0.30 ± 0.02*</td>
<td>0.22 ± 0.04*</td>
<td>0.12 ± 0.02*</td>
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<td>AK activity, IU/mg dry heart wt</td>
<td>0.14 ± 0.02</td>
<td>0.17 ± 0.02</td>
<td>0.13 ± 0.02</td>
<td>0.11 ± 0.01</td>
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<tr>
<td>Protein content, mg/mg dry heart wt</td>
<td>0.88 ± 0.08</td>
<td>0.90 ± 0.05</td>
<td>0.80 ± 0.05</td>
<td>0.90 ± 0.05</td>
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<td>n</td>
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<td><strong>Isolated mitochondria experiments</strong></td>
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<td>ADP stimulated (1 mM)</td>
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<tr>
<td>ADP:O</td>
<td>2.34 ± 0.08</td>
<td>2.24 ± 0.17</td>
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<tr>
<td>State 3 V̇O₂</td>
<td>497 ± 52</td>
<td>553 ± 81</td>
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<td></td>
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<tr>
<td>State 4 V̇O₂</td>
<td>52 ± 8</td>
<td>62 ± 15</td>
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<tr>
<td>RCR</td>
<td>10.0 ± 1.3</td>
<td>9.6 ± 1.1</td>
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<td>ATP stimulated + 20 mM creatine</td>
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<tr>
<td>0.5 mM ATP</td>
<td>126 ± 4</td>
<td>98 ± 4*</td>
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<tr>
<td>1.0 mM ATP</td>
<td>344 ± 5</td>
<td>233 ± 38*</td>
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<tr>
<td>1.5 mM ATP</td>
<td>451 ± 15</td>
<td>334 ± 46</td>
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<tr>
<td>CK mitochondrial activity, IU/mg mitochondrial protein</td>
<td>1.15 ± 0.12</td>
<td>0.026 ± 0.004*</td>
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Data represent means ± SE; n = no. of hearts. IA, iodoacetamide-treated hearts; CK, creatine kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; AK, adenylate kinase. State 3 V̇O₂ and state 4 V̇O₂, respective respiration rates (in natoms oxygen·mg mitochondrial protein·min⁻¹); RCR, respiratory control ratio (state 3/state 4 respiration); CK mitochondrial activity, CK activity in isolated mitochondria suspension. Isolated mitochondria were added to 2-ml reaction vessel at a protein concentration of 0.5 mg/ml. O₂ consumption rate (in natoms oxygen·mg mitochondrial protein·min⁻¹) was measured in mitochondria stimulated with 20 mM creatine and varying concentrations of ATP. *P < 0.05 vs. control hearts.
hearts was not significantly higher than in controls, and even the extra rise in t_{transport} (representing ~0.7 s) cannot explain the 3-s difference in t_{mito} between these groups. The pretreatment correction times for t_{APP} and t_{init} during heart rate steps between 135 and 220 beats/min were similar in all groups and were 0.6–1.2 s and 0.1–0.3 s, respectively. These values were not significantly changed over time in the control hearts or affected by IA treatment at any dosage.

Although the O$_2$ consumption of the whole heart was marginally reduced by IA infusion (see Fig. 2), the increases in MVO$_2$ for steps from 135 to 160, 190, and 220 beats/min (11, 20, and 29%, respectively) were not significantly different between or within groups before and after IA. When arterial O$_2$ concentration was decreased by 8.3 ± 0.4%, MVO$_2$ did not decrease significantly at 135 beats/min (0.7 ± 0.8%) but was 2.1 ± 0.5% lower at 220 beats/min (P < 0.05). These changes were similar in all groups and were not significantly altered after CK inhibition. From these results we chose to use the transport time derived from the arterial concentration step at 135 beats/min, at which MVO$_2$ was stable during supply reduction, to correct the vPO$_2$ response time (t$_v$) for transport delay.

**DISCUSSION**

The present study was designed to test the acute effects of graded CK inhibition on the response times of myocyte energy signaling during rapid submaximal workload shifts in the intact rabbit heart. The results demonstrate accelerated signal speeds to mitochondria for increased oxidative phosphorylation with progressively higher blockade of CK activity. This finding was obtained without changes in mitochondrial oxidative capacity or substrate limitation. However, the observed reduction in contractile reserve at high workloads in previous (12, 25) and present studies suggests that although energy transfer by CK from oxidative phosphorylation can be effectively bypassed (given the quick mitochondrial response), ADP and ATP buffering near ion pumps and myofibrillar ATPases by CK is essential during enhanced myocardial performance.

The specificity of IA to block CK activity must be critically examined. Although IA was previously used in isolated rat heart studies as a specific CK inhibitor (9, 10, 12, 24), it is known to cause alkylation of other sulfhydryl enzymes, as shown by GAPDH inhibition in the present study and by others (9, 24). However, glycolytic flux (24), AK (Ref. 10 and present study), and myofibrillar ATPase (10) activities remain unaffected by IA. Moreover, Tian and Ingwall (25) observed some heterogeneity in the blockade pattern of myocardial CK isoenzymes after infusion of IA. In the present study we also observed a small but nonsignificant difference in the inhibition rates of mitochondrial and total tissue CK activities. Therefore, without the current availability of isozyme-specific CK inhibitors and as yet no way to measure our dynamic oxidative response times in the CK "knockout" mice (29–31), we conclude that IA infused slowly at set concentrations is an effective means of studying the acute effects of CK inactivity in isolated hearts. Importantly, acute inhibition of CK with IA has the advantage over knockout of a gene in that no compensatory mechanisms involving altered gene expression play a role (29).

Effects of CK inhibition on contractile function. IA did have significant effects on contractile function during infusion of the higher concentrations, similar to those seen in rat hearts (25). Prominent was the 53% rise in EDP, which presumably reflects elevated free ADP concentration. Indeed, Tian et al. (24) recently used IA to cause a threefold increase in EDP that was matched by ADP levels without changing ATP concentration, P$_i$ concentration, or intracellular pH. The ability of hearts to still maintain low-to-moderate cardiac workloads after severe reductions in CK reaction velocity has now been demonstrated by a number of groups using chemical inhibition of CK (9, 10, 24) and animals fed with Cr pool analogs (22).

Loss of contractile reserve in response to inotropic (10, 25) or pressure-volume (9, 12) work stimulation is also a finding common to hearts treated with IA. Reductions of 35–72% in functional reserve in rat hearts with <10% of CK activity have been observed during high-calcium perfusion (10, 24). The modest (but significant) 10% decreases in contractile reserve we observed in heart groups with 3 and 6% remaining CK activity perhaps reflects the higher energy cost of contraction (as used in Refs. 9, 10, 12, 25) versus rate work as used in the present study (2).

CK activity and response times. ADP-stimulated O$_2$ consumption in isolated mitochondria indexed as ADP:O, respiratory control ratios, and maximal state 3 V$_O2$ were unchanged in IA-treated hearts (Ref. 10 and present study). Moreover, we recently reported that up to 50% reductions in mitochondrial aerobic capacity do not change the response times (t$_{mito}$) in normoxic rabbit hearts with full CK activity (4, 36). Furthermore, in the
present experiments no differences in the response of hearts to small reductions in arterial O2 concentration were observed after inhibition of CK, suggesting unaltered sensitivity to O2 supply.

The progressive shortening of t\textsubscript{mito} paralleled the level of inhibition of CK activity such that in hearts with only 3% CK, energy signaling in response to a heart rate step was \( \sim 3 \) s faster than in controls. This graded effect on t\textsubscript{mito} is similar to the effect on contractile reserve seen during progressive CK inhibition in the present study and in rat hearts (25). Contractile reserve and t\textsubscript{mito} were maintained until CK velocity fell below 15% of control, and then they fell sharply, highlighting the functional reserve of the CK system. During myocardial stunning secondary to ischemia and hypoxia t\textsubscript{mito} was increased by \( \sim 40\% \) (36). The hypothesis that this was caused by inhibition of CK by reactive oxygen species is dispelled by the findings in the present study. With the quicker response times in the current experiments being independent of any changes in mitochondrial oxidative capacity, we suggest that cytosolic ATP synthesis to hydrolysis coupling is accelerated in the absence of CK buffering. This finding contradicts the essential transport role of the CK/PCr shuttle (3, 11, 33) and the concept of PCr/Cr rather than ATP/ADP metabolite signal transduction (18, 35), instead supporting the concept of cytosolic CK being a high-capacity temporal buffer localized in the myofibrils and the cytosol. CK may instead act as a high-capacity temporal buffer (15, 23) that normally delays the response of mitochondria to rapid energy demand increases. Such a system would allow temporary cytosolic uncoupling of energy production from its utilization, especially during large cardiac workload transitions (20).

The increase in response times over time in control hearts (33 ± 8%) is similar to that observed in the previous study of Zuurbier and van Beek (36) using an analogous heart preparation and time protocol. Although a clear explanation cannot be given, we speculate that such factors as interstitial edema, intracellular swelling, and the potential for oxygen free radical damage in the high-P\textsubscript{o2} crystalloid-perfused hearts may contribute to increased intracellular diffusion distances and inhibition of cytosolic enzymes, respectively. In addition, the exhaustion of endogenous substrates and the consequent transition and reliance on exogenous supplies over time may also play a role, because we observed variations in response times when exogenous substrates were varied among glucose, pyruvate, and lactate (27). When pyruvate (2 mM) was added in addition to glucose (unpublished pilot experiments, n = 6), the increase in t\textsubscript{mito} over time in control hearts was reduced. Because of this gradual change in response time, the effect of IA infusion was carefully compared with a time-control group.

Alternative mechanisms of faster energy signaling. An explanation for the decrease of t\textsubscript{mito} in CK-inhibited hearts is that higher ADP levels alter diffusion. Given that PCr is decreased quickly during upward steps in heart rate in our preparation (7), CK action may delay the local increase in ADP. However, alternative explanations should be considered.

A possible reason for the faster t\textsubscript{mito} could be that as CK is blocked, energy transfer via the AK shuttle, which is normally low, is upregulated (6, 21). In rat diaphragm in which 1-fluoro-2,4-dinitrobenzene was used to block CK activity by 98%, the contribution of AK flux to the total phosphoryl transfer (\( \sim 7\% \)) increased reciprocally (as CK flux fell) to levels approaching the preinhibition CK flux (\( \sim 88\% \)) (5). Our results and those of others (10) have shown no increase in in vitro AK activity in hearts with chemically inhibited CK activity. Given that the total phosphate group transfer was still decreased in these rat diaphragm experiments, indicating incomplete compensation by the AK shuttle, it is unlikely that enhanced AK flux would explain the dramatically quickened energetic signaling speeds observed in our studies in hearts.

Further contributions to the observed faster response times to rapid ATP hydrolysis may come from glycolytic buffering. We recently suggested that glycolysis may also play an important energy transfer function in the myocyte and that glycolytic buffering, especially in and near myofibrils, may also retard the oxidative signal to the mitochondria (26, 27); the work of Dzeja et al. (5) points in a similar direction. We observed a 68% reduction in activity of the key glycolytic enzyme GAPDH at the highest level of CK inhibition (97%), consistent with previous IA infusion experiments in rat hearts (9, 24). Recent work by the group of Ingwall (10, 24) in IA-treated rat hearts showed no \( ^{31} \)P NMR-detectable increases in glycolytic intermediates, and measured glycolytic rate was unchanged in hearts with only 20% GAPDH activity remaining, which reflects the fact that GAPDH activity normally exceeds glycolytic rate \( \sim 50\)-fold. Furthermore, in a series of pilot experiments (n = 6, results not shown), we added pyruvate (2 mM) to the Tyrode buffer before inhibition of CK with 0.4 mM IA. The t\textsubscript{mito} measurements again decreased (independent of the test heart rate) by \( \sim 44\% \) from pretreatment levels (3.4 ± 0.5 to 1.9 ± 0.4 s, P < 0.05), thereby removing the possibility that restricted glycolysis confounded our original results.

In conclusion, we found that graded inhibition of CK by IA infusion in isolated rabbit hearts caused an increase in the cytosolic signaling speed for ATP synthesis, indicated by a quicker response of mitochondrial O2 consumption during rapid pacing-induced workload jumps. The acceleration in the response time of oxidative phosphorylation was dose dependent and not related to changes in mitochondrial oxidative capacity or changes in O2 diffusion or transport delays to the myocyte. These results go against the theory of cytosolic CK operating as an essential energy transport shuttle. CK may instead act as a high-capacity temporal buffer localized in the myofibrils and the cytosol effectively slowing the signal to the mitochondria. The loss of this CK-mediated buffering of ADP and P, near the myofibrils and ion pumps is reflected by decreased contractile reserve of hearts during rapid increases in cardiac performance.
QUICKER ENERGY SIGNALING IN CK-INHIBITED HEARTS

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