Energetics of heart muscle contraction under high K perfusion: verapamil and Ca effects

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Consolini, Alicia E., María T. Márquez, and Jorge E. Ponce-Hornos. Energetics of heart muscle contraction under high K perfusion: verapamil and Ca effects. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H2343–H2350, 1997.—Tension-dependent (TDH) and tension-independent heat (TIH) release were measured during single isovolumetric contractions in the arterially perfused rat ventricle. Under perfusion with 7 mM K-0.5 mM Ca, TDH showed only one component (H1 and H2) of short evolution (similar to the classically identified activation heat) and one component (H4) of long duration (dependent on mitochondrial respiration). Under 25 mM K, TIH components (i.e., H1, H2, and H4) increased with the increase in extracellular Ca concentration ([Ca]o) from 0.5 to 4 mM, and H3 correlated with pressure at all [Ca]o, with regression parameters similar to those observed under 7 mM K. Under 25 mM K-2 mM Ca, peak pressure development (Pp), H1, H2, and H3, plotted against the number of beats under 0.4 µM verapamil, exponentially decreased, but H4 decreased to 5.5 ± 2.9% in the first contraction and remained constant thereafter. Under hypoxia, P, H1, H2, and H3 progressively decreased for about six contractions, but H4 was not detectable from the second contraction. The results suggest that increasing extracellular K concentration decreases mitochondrial activity mainly by increasing energy expenditure related to a Ca-dependent (verapamil-sensitive) mitochondrial activity that is not related to force generation.

an increased extracellular K concentration ([K]o) has been used to prevent spontaneous contractions for the measurement of basal metabolism (17, 29). In surgery, high [K]o has been included in cardioplegic solutions to safeguard the heart from damage (11). On the other hand, it has been shown (23) that, under quiescent conditions, increasing [K]o increases steady energy expenditure caused by an increase in the Na-K pump activity (23). Therefore, it was of interest to determine whether high K perfusion also affects the economy of a contraction. The economy of isometric force development can be altered by affecting two major groups of processes, namely tension-dependent and tension-independent processes (4, 12, 13). To minimize the energy contribution by tension-dependent processes, the energy released by tension-independent processes has been classically studied by diminishing force development. Several methods have been used to achieve that goal, such as shortening the resting length of the muscle (12), exposing the muscle to a hyposmotic medium (19), or quick-releasing the muscle during the latency period (13). More recently, four components of heat released (H1, H2, H3, and H4) were simultaneously measured in a single contraction (22). The most relevant aspect of these measurements is that the tension-independent heat (TIH) was evaluated simultaneously with the tension-dependent heat (TDH) component (H3) in the presence of pressure development (22). TIH was further divided into two fractions (H1 and H2) of short evolution (similar to the fraction classically identified as the activation heat) and another one of long duration (H4) that showed a high dependence on mitochondrial respiration (22). With the use of this approach for simultaneous measurement of TIH and TDH, the present work shows that maintenance of isovolumic pressure development under high [K]o perfusion induces an overall decrease in muscle economy. The effect can be mainly attributed to the increase in basal heat production and to an increase in the activity of a tension-independent, oxygen-dependent mechanism that is triggered by Ca. Furthermore, the present results also show that, under these high K conditions, the contractile economy decreases as [Ca]o increases.

METHODS

Biological preparation. Twenty-four Wistar rats of either sex, weighing 200–250 g, were reserpinized (5 mg/kg; Ciba Geigy) 24 h before death. The animals were heparinized (2,000 U) and anesthetized with a pentobarbital sodium overdose (23). The beating hearts were rapidly excised, and retrograde perfusion by the Langendorff method was initiated with control perfusate at room temperature (20–24°C). Both right and left atria and right papillary muscles were dissected from the heart. Also, a small cut in the septal wall, close to the aorta, was performed to prevent spontaneous contractions. A latex balloon was placed into the left ventricle, and the muscle was mounted in a Kel-F frame between two stainless steel hooks. After cannulation and mounting, the muscle was placed in the inner chamber of a calorimetric system. The latex balloon was connected to a Statham P23 Db pressure transducer so that pressure developed during isovolumic contractions could be measured. At the end of each experiment, the tissue was removed from the calorimeter, weighed in a preweighed vial, and dried at 110°C to constant weight so that the water content could be calculated. The average water content in the present experiments was 81.7 ± 0.49% (n = 24). Unless otherwise indicated, results reported in the present work are quoted per gram wet weight.

Solutions. The heart muscle was perfused at a constant rate (5 ml/min) with a solution (control) containing (in mM) 1 MgCl2, 100 NaCl, 0.5 NaH2PO4, 7 KCl, 0.5 CaCl2, 25 NaHCO3, 36 sucrose, and 6 dextrose. Sucrose was replaced by 18 mM KCl for the high-K perfusate (25 mM K). The solutions were bubbled with 95% O2-5% CO2 or 95% N2-5% CO2 for the hypoxic experiments to achieve a pH of 7.3–7.4. In those experiments...
in which Ca concentration in the perfusate was changed, no corrections for changes in osmolarity or ionic strength were performed. Verapamil (Hoescht) was diluted in Krebs solution from a 1 × 10^{-3} M solution the same day of the experiment.

Mechanical and heat measurements. The technique for on-line measurement of heat production and mechanical activity of isolated heart muscle has been described previously in detail (10, 24). Briefly, the calorimeter was submerged in a constant-temperature bath. The temperature of the calorimeter bath was controlled with a cooling-heating bath (±0.003°C) in which the perfusate was also equilibrated. Calorimeter calibration was accomplished by passing a 2.1-kHz sine wave through the muscle by means of the stimulating electrodes (24). The present calorimeter uses two insulated ceramic modules (Melchor Thermoelectrics) with a total of 254 thermosensitive junctions (22). The minimum output of the thermosensitive units recorded in the present experiments was >10 µV, whereas the electrical noise was 1 µV at a maximum gain (1 µV/mm). With this method, it was possible to continuously and simultaneously record left intraventricular pressure, its first derivative, perfusion pressure, and rate of heat production (H). Both mechanical and heat outputs were recorded on a Grass SD 5 four-channel recorder. In some experiments, heat production was also logged by an analog-to-digital converter (DT 2808, Data Translation) into an AT-386 desk computer. Data acquisition frequency varied from 1 to 40 data points per second. The mechanical parameters considered for this study were maximal intraventricular pressure development (P), intraventricular pressure-time integral (PTI) measured as the area under the pressure development signal, and maximal rates of contraction (+P) and relaxation (−P). The whole contraction was divided into three periods as follows: t0p, time to peak pressure measured from the start of contraction to t0p; t1p, time from t0p to −P; and t2p, time from −P to the end of contraction.

Once the muscle was placed in the inner chamber of the calorimeter, a 60-min equilibration period with control solution was allowed to elapse before any experimental intervention. The muscle was stimulated by means of the stimulating electrodes (24). The present experiments was studied with the sign test (9). Statistical significance between two different correlation coefficients was estimated as described elsewhere (9). The comparison between fitted curves followed by a nonparametric Mann-Whitney ranking test was applied (27). Regression analysis was performed with the use of a nonlinear regression technique running on an AT-386-compatible desk computer (22). The difference between the estimated and the hypothetical value (i.e., estimated slope against 1, correlation coefficients, and zero absissae values against 0) was analyzed as described elsewhere (9), and the statistical significance was settled at P < 0.05. Systematic deviations of the fitted curve from the data points were studied with the sign test (9). Statistical significance between two different correlation coefficients was estimated as described elsewhere (9). The comparison between fitted curves obtained with different numbers of terms (for a given set of data points) was performed with the Fisher’s test (9).

RESULTS

Under control perfusion (7 mM K-0.5 mM Ca), rest heat production averaged 4.16 ± 0.15 mW/g. Figure 1 shows that, after 5-min periods of quiescence, maximal pressure development (P) averaged 42.5 ± 2.9 mN/mm² and active heat (Hₐ) averaged 31.6 ± 3.6 mJ/g (n = 14). In all experiments, the power curve obtained under control perfusate was fitted to four heat components (Fig. 2A). The mean values obtained were 2.2 ± 0.3, 2.3 ± 0.3, and 16.9 ± 1.8 mJ/g for the TDH release (H₃), respectively; and 8.9 ± 0.7 mJ/g for the TDH release (H₃).

Effects of 25 mM K. As previously described (23), changing the perfusate from 7 mM K-0.5 mM Ca to 25 mM K-0.5 mM Ca induced a transitory increase in Hₐ.
(+1.46 ± 0.42 mW/g). This increase was followed by a decrease in resting heat values to a new steady level that remained higher (+1.17 ± 0.33 mW/g; n = 14; P < 0.01) than control resting heat (4.16 ± 0.15 mW/g). Under this new resting condition, P and H₄ decreased to ~20% of their respective values under control perfusate (Fig. 1). Maximum rate of contraction (+P) and relaxation (−P) also decreased. To investigate whether the decrease in +P and −P were associated with the decrease in P, the ratios between +P or −P and P were studied. Whereas the +P-to-P ratio remained unchanged (8.7 ± 0.6 vs. 9.7 ± 0.9 s⁻¹; n = 8; not significant), the −P-to-P ratio significantly increased from 3.1 ± 0.3 to 4.7 ± 0.7 s⁻¹ (P < 0.05). Neither time to peak pressure (tPP) nor the last part of the relaxation period (tR2) changed with the increase in [K]o. On the other hand, the first period of relaxation (tR1) significantly decreased from 0.26 ± 0.02 to 0.15 ± 0.02 s (P < 0.01). The energy released by a contraction under 25 mM K-0.5 mM Ca perfusate was always decomposed into only three components (H₁, H₂, and H₃). As shown in Figs. 2–4, all three components of heat released under 25 mM K-0.5 mM Ca were smaller than those obtained under control perfusate.

Effects of increasing [Ca]₀ under 25 mM K. To compensate for the negative inotropic effect of 25 mM K, [Ca]₀ was increased from 0.5 mM to 4.0 mM. The experimental sequence of altered [Ca]₀ was 0.5, 1.0, 2.0, and 4.0 mM. As shown in Fig. 1, increasing [Ca]₀ under 25 mM K perfusate increased P (up to 85% of control values) and H₄ (up to 830% of control, under 4 mM Ca). The +P-to-P ratios at 1, 2, and 4 mM Ca did not change with the changes in [Ca]₀, and the pooled data (n = 36) averaged 9.6 ± 0.5 s⁻¹. The −P-to-P ratio (which was increased by 25 mM K-0.5 mM Ca) was not altered by increasing [Ca]₀, and the pooled data for 1, 2, and 4 mM Ca averaged 4.5 ± 0.3 s⁻¹. Similarly, under 25 mM K, tR₁ remained unchanged with changes in [Ca]₀ (pooled data average: 0.14 ± 0.01 s; n = 36), but it was shorter (P < 0.05) than the tR₁ observed under 7 mM K (0.26 ± 0.02 s; n = 14). The second period of relaxation (tR₂) was significantly prolonged only under 25 mM K-4 mM Ca (1.44 ± 0.26 vs. 0.53 ± 0.05 s for 25 mM K-4 mM Ca and 7 mM K-0.5 mM Ca, respectively; P < 0.01). This prolonged relaxation was not enough to significantly increase PTI when [Ca]₀ was increased from 2 to 4 mM Ca.

The increase in [Ca]₀ under 25 mM K increased all four components of heat production (Figs. 2–4). H₁ significantly increased when [Ca]₀ was raised from 0.5 to 1 mM under 25 mM K media and remained unchanged and similar to the control (2.2 ± 0.3 mJ/g)
between 1 and 4 mM Ca (Fig. 3A). H2 significantly increased from 0.5 to 4 mM Ca (Fig. 3A), reaching a value (4.0 ± 0.5 mJ/g) higher (P < 0.001) than that measured under control perfusate (2.3 ± 0.3 mJ/g). As reported for 7 mM K (22), H3 varied proportionally with P and PTI. Because at the various [Ca]o, no significant differences among their slopes were found, the data were pooled. The correlation for 44 data points yielded a straight relationship with r > 0.74 (P < 0.001) (Fig. 4). The tension-independent, oxygen-dependent heat component H4 was absent at 0.5 mM Ca-25 mM K conditions (see Fig. 2B). On the other hand, it significantly increased as [Ca]o was raised (Fig. 3B), reaching a value (253 ± 36 mJ/g) of ~15 times that observed under 7 mM K-0.5 mM Ca (16.9 ± 1.8 mJ/g). Under 1 mM Ca, H4 showed similar magnitude and kinetic characteristics to those observed under control perfusate. At 2 and 4 mM Ca, H4 increased exponentially for >80 s after the mechanical event was over (Fig. 2C). The exponential increase in H4 under 2 or 4 mM Ca-25 mM K was further supported by the fact that when data from the calorimetric output (observed after 60 s of contraction) were divided by the diffusional term of Eq. 1, an exponential function was consistently observed.

Effects of verapamil under 25 mM K-2 mM Ca. As shown in Effects of increasing [Ca]o under 25 mM K, all four heat components released under high-K perfusate were dependent on [Ca]o. To investigate the relationship of the heat components with Ca influx via Ca channels, five experiments were done in which the effect of 0.4 µM verapamil was tested during 25 mM K-2 mM Ca perfusion. Verapamil was added during quiescence in the presence of 25 mM K-2 mM Ca. About 2 min later, two or three electrical stimuli (5 min apart) were applied to each muscle. Verapamil induced a decrease in P which was dependent on the period elapsed in the presence of the drug under 25 mM K (Fig. 5D). It is of interest that the effects of verapamil were independent of the number of isolated contractions performed (Fig. 5). No changes were found in resting pressure or P˙-o or P˙-to-P ratios. When P, H1, H2, and H3 values obtained in the presence of verapamil were plotted against time, all three heat components exponentially decreased with similar time constants (Fig. 5). On the other hand, H4 decreased with verapa-
mil treatment to 6.7 ± 3.2% (n = 12, P < 0.05 against 0) of the value under 25 mM K-2 mM Ca perfusate independent of the duration of the verapamil treatment.

Severe hypoxia under 25 mM K-2 mM Ca. It has been suggested (22) that under control perfusate, H3 was associated with mitochondrial activity. To test whether the magnitude of H3 observed under high [K]o perfusion was also oxygen-dependent, in five experiments (after at least 30 min under 25 mM K-2 mM Ca-perfusion) the oxygen of the perfusate was removed by switching to a 95% N2-5% CO2 perfusate. Three minutes after the hypoxic perfusate was started, five or six pulses separated by intervals of 5 min were applied and their mechanical and heat outputs recorded. As shown in Fig. 6, the decrease in H3 through the successive hypoxic contractions was mostly caused by a decrease in H4 (which fell to 30% of its original value in the first hypoxic beat and disappeared in the second beat). In contrast, the decreases in P, H1, H2, and H3 with the successive hypoxic beats were similar. As shown in Fig. 6, A and B, these components remained present in each muscle for at least five contractions. The energy released as H3 was linearly correlated with P under hypoxic conditions (slope: 0.17 ± 0.02 mJ·mN⁻¹·mm²·g⁻¹; intercept not different from zero: 0.55 ± 1.2 mJ/g; r = 0.7988). The slope for H3 versus P in hypoxia was about 80% of that observed for aerobic conditions under 25 mM K-2 mM Ca perfusion (slope: 0.21 ± 0.06 mJ·mN⁻¹·mm²·g⁻¹). The paired differences between the H3-to-P ratios in hypoxic and aerobic conditions averaged -0.024 ± 0.009 mJ·mN⁻¹·mm²·g⁻¹ (n = 28, P < 0.01). No changes were found in resting intraventricular pressure or in +P- or -P-to-P ratios during the hypoxic period studied (<35 min).

DISCUSSION

It has been shown that the energy released by a single contraction can be decomposed into TDH (H3) and TIH components (22). Two of the TIH components (H1 and H2) were related to a fraction classically identified as activation heat (mainly myofilament calcium-binding and calcium-removal processes, respectively). Because of its oxygen dependency, a third fraction of TIH was associated with the mitochondria (22). Increasing [K]o induced a caffeine-sensitive transitory increase in H4 (23). It was suggested that the high [K]o could be depleting the sarcoplasmic reticulum (SR), leaving less Ca available for the twitch (23). This suggestion agrees with the observed fall in P under 25 mM K-0.5 mM Ca perfusate and with the decreased H1. Because high [K]o perfusion induces an increase in the Na-K pump activity (23), a decrease in intracellular
exchanger and the one ATP hydrolyzed for every three Na removed via the Na-K pump) and two Ca per ATP via the SR Ca pump (6, 21), an increase of the activity of Na/Ca exchanger over the SR Ca pump should result in an increase in energy expenditure.

Because H₃ is the only pressure-dependent energy release component of the contraction, the ratio between P and H₃ can be used as a measure of the isometric economy (22). When both P and H₃ are expressed in the same units [using 1.05 as the density of the muscle (15)], the ratio between them is dimensionless. This ratio would be a measure of the isometric heat coefficient, which is a useful index of contraction economy (4, 19). Under 25 mM K perfusate, the P-to-H₃ ratio (~4.3) calculated from the inverse of the slope of the plot shown in Fig. 4A was independent of changes in [Ca]₀. Furthermore, it was similar to that calculated from single contractions under 7 mM K-0.5 mM Ca perfusate (~3.9). The P-to-H₃ (~4.3) and PTI-to-H₃ (~1.44 s⁻¹) ratios under 25 mM K were similar to those reported for rat ventricles perfused under 7 mM K-0.5 mM Ca (~4.2 and 1.5 s⁻¹, respectively) (22). The P-to-H₃ ratio was also similar to that calculated from the TDH of rabbit myocardium (~3.6) (19). The absence of changes in these ratios would indicate that varying [K]₀ and [Ca]₀ does not affect the economy of force development or that of force maintenance. Hypoxia also increased the P-to-H₃ ratio (to 5.6) under 25 mM K conditions, indicating as previously suggested under 7 mM K (22) the existence of a heat fraction related to recovery metabolism in H₃.

It is clear from Fig. 3 that the increased H₄ is mainly caused by an increased H₄. As previously shown for 7 mM K (22), under 25 mM K perfusate H₄ is a pressure-independent and oxygen-dependent fraction of energy. The fact that under O₂ deprivation H₄ disappeared even under conditions in which P was scarcely affected (see the second hypoxic beat in Fig. 6) indicates that H₄ should be coupled to mitochondrial respiration. A range of evidence indicates that whereas P and H₄ are both related to [Ca]₀ under 25 mM K, there seems to be no direct relationship between them. For instance, whereas P was saturated at 2 mM Ca, H₄ increased with a further increase in [Ca]₀ to 4 mM (see Fig. 3B). In addition, the fact that under 25 mM K-0.5 mM Ca H₄ could not be detected suggests that the processes associated with H₄ might have a higher Ca threshold than P. Furthermore, whereas H₄ was strongly inhibited by verapamil in a time-independent fashion, the effects of verapamil on P, H₁, H₂, and H₃ were time dependent and all four parameters were affected with a similar time constant (see Fig. 6). These results suggest that whereas P, H₁, H₂, and H₃ might have a common dependence on Ca channels, H₄ could be related to another verapamil-sensitive site. In this connection, it has been shown that verapamil inhibits the mitochondrial Na/Ca exchanger (7, 30), which participates in the mitochondrial Ca transport cycle (8, 14). The differential sensitivity of H₄ to verapamil and the fact that it is altered by changes in [Ca]₀ and is coupled to mitochon-
drial respiration suggest a Ca-related mitochondrial activity. Furthermore, the time course of $H_4$ (developed for > 80 s after the twitch) indicates that this process is active even after the contraction-relaxation cycle has finished. In this connection, it is known that cytosolic Ca could trigger either an increase of oxidative phosphorylation in response to ATP-consuming processes (5) or a Ca cycling through mitochondrial membrane coupled to respiration (6, 8, 14, 16). Therefore, the presence of $H_4$ (even under control perfusate) suggests that the mitochondria participate in the homeostasis of a Ca fraction in response to a contraction but that this Ca fraction is different from that involved in the development of pressure.

It is well known that hypoxia decreases mechanical activity in general and P in particular. The decrease in P has been attributed to a number of processes such as 1) a decrease in ATP or creatine phosphate content (20, 26); 2) intracellular acidosis caused by glycolytic lactate and H+ accumulation (2); or 3) a decrease in Ca influx or Ca release (1, 20). The effects of hypoxia on P, $H_1$, $H_2$, and $H_3$ under 25 mM K perfusate observed in the present work were more marked than under control perfusate (22). Nevertheless, resting pressure and relaxation times were not altered by hypoxia, suggesting that at least ATP levels near the myofilaments and Ca pumps were not significantly affected. It has been shown that acidosis by itself does not reduce the cytosolic peak of aequorin (3), indicating that the amount of Ca released should remain approximately constant. In line with this finding is the fact that $H_1$ and $H_2$ reportedly (22) did not decrease during the fall in P observed under hypoxic control perfusion. On the other hand, under 25 mM K conditions, the progressive fall in $H_1$ and $H_2$ suggests that hypoxia could have decreased the availability of Ca for myofilaments, which in turn could have a further effect on P. Therefore, whereas the negative inotropism found under control perfusion could be ascribed to acidosis, the larger decrease in P observed under high-K media might be related to an additional effect on cytosolic Ca. This interpretation is also supported by the fact that a decreased 47Ca uptake has been found in rabbit intraventricular septum during hypoxia (20) and that a shortening in action potential duration is induced by anoxia in rat ventricular myocytes (28). Because high K perfusion induces a Ca depletion from SR (18, 23), P should be more dependent on Ca influx under high K than under 7 mM K. Consequently, an effect of hypoxia on Ca current should be more noticeable on cytosolic Ca and P under 25 mM K than under 7 mM K conditions. In summary, increasing $[K]_o$ decreases contractile economy mainly by increasing energy expenditure related to a long-duration, Ca-dependent, and verapamil-sensitive mitochondrial activity different from that related to force generation. Therefore, the increase in energy expenditure is pressure independent and likely caused by an increase in the energy expenditure for Ca sequestration and for a Ca-dependent increase in mitochondrial activity.

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