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

ALICIA E. CONSOLINI, MARÍA T. MÁRQUEZ, AND JORGE E. PONCE-HORNOS
Instituto de Investigaciones Cardiológicas, Facultad de Medicina y Cátedra de Biofísica, Facultad de Odontología, Universidad de Buenos Aires-Consejo Nacional de Investigaciones Científicas y Técnicas, 1122 Buenos Aires, Argentina

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) of short evolution (similar to the classically identified activation heat) and one component (H2) of long duration (dependent on mitochondrial respiration). Under 25 mM K, TIH components (i.e., H1, H2, and H3) 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 (P), 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 contractile economy mainly by increasing energy expenditure related to a Ca-dependent (verapamil-sensitive) mitochondrial activity that is not related to force generation.

Methods

Biological preparation. Twenty-four Wistar rats of either sex, weighing 200–250 g, were reserpinized (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. A latex balloon was placed into the left ventricle, and the muscle was mounted in a Kef 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

0363-6135/97 $5.00 Copyright © 1997 the American Physiological Society H2343
in which Ca concentration in the perfusate was changed, no corrections for changes in osmolarity or ionic strength were performed. Verapanil (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 5D 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: t₀, time to peak pressure measured from the start of contraction to P; t₁, time from P to −P; and t₂, 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 solu-

Recommended Ca and energetic of heart beat under high K

where A₀ = (μ4π⁻²β⁻³)tan[(μ4π⁻²β⁻¹)²]; μ is the cooling rate constant of the calorimeter; β is the diffusion delay constant; A₁ = 1/[2(1 + 1)]F [−(1 − (2 + 1)μ⁻¹)]; c₁ = (2 + 1)μ; t is time; and H₀ represents the applied power. On the other hand, if the released power grows for longer periods than the integration time of the calorimeter, H in Eq. 1 becomes a function of time. To test whether this time dependency could be obtained from the calorimeter output, power was released in the calorimetric chamber following either an exponential (4 experiments) or a linear function of time (4 experiments). Regardless of how power was generated, each calorimetric response was fitted by two diffusional equations similar to Eq. 1. In one of them, H₀ in Eq. 1 is equal to H₁(1 − e⁻ᵃ⁽dots⁾), and in the other equation, H₀ = k₄t, where φ and k are the exponential and linear rate constants, respectively. The fitting procedure was applied 1) to the whole curve (i.e., from the time at which power was applied to the time at which power was switched off) and 2) to selected periods (from time 0) of that curve. The fitted parameters remained constant only if the curve was fitted with the function under which power was applied. When an exponential fit was used for data processing, the area under a linear growth curve was obtained by a linear function of time and was used as a measure of muscle stability. Resting pressure was increased in steps of 1–2 mN/mm² with the pressure measured from the start of contraction to P; t₁, time from P to −P; and t₂, 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 5-V, 5-ms square pulses from a Grass SD 9 stimulator in control medium and by ≤15-V, 15-ms stimulus in high-K media. The stimulus contribution to the heat released was <1% of that released by a contraction. A muscle was accepted for study if, during the 60-min equilibration period under control perfusate at 25°C, the electrical stimulation was stopped and the resting heart rate (Hᵣ) was recorded. The pressure and heat production from single twitches (5 min apart) were recorded at a chart speed of 25–50 mm/s.

Heat signal analysis. Active heat per beat (Hₐ; the fraction released above Hᵣ by a contraction) was calculated as the integral of the calorimetric output (Hᵣ) versus time (22, 25). The various components associated with this H₀ were calculated as described elsewhere (22). Briefly, when the power applied was either maintained constant or interrupted before the integration time of the calorimeter, Hᵣ for the period during which the power was applied can be described by the following equation (22)

\[ Hₐ = H₀ \left(1 - Aₑ^{−βt} - 8r^{-2} \sum_{i=0}^{∞} Aᵢₑ^{−μi}t \right) \] (1)

RESULTS

Under control perfusion (7 mM K-0.5 mM Ca), resting 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 TΠh components (H₁, H₂, and 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 Hᵣ,
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 H_a 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 (t_PP) nor the last part of the relaxation period (t_R2) changed with the increase in [K]_o. On the other hand, the first period of relaxation (t_R1) 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 (H_1, H_2, and H_3). 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]_o under 25 mM K. To compensate for the negative inotropic effect of 25 mM K, [Ca]_o was increased from 0.5 mM to 4.0 mM. The experimental sequence of altered [Ca]_o was 0.5, 1.0, 2.0, and 4.0 mM. As shown in Fig. 1, increasing [Ca]_o under 25 mM K perfusate increased P (up to 85% of control values) and H_a (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]_o, 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]_o, and the pooled data for 1, 2, and 4 mM Ca averaged 4.5 ± 0.3 s⁻¹. Similarly, under 25 mM K, t_R1 remained unchanged with changes in [Ca]_o (pooled data average: 0.14 ± 0.01 s; n = 36), but it was shorter (P < 0.05) than the t_R1 observed under 7 mM K (0.26 ± 0.02 s; n = 14). The second period of relaxation (t_R2) 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]_o was increased from 2 to 4 mM Ca.

The increase in [Ca]_o under 25 mM K increased all four components of heat production (Fig. 2–4). H_1 significantly increased when [Ca]_o 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]₀, 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]₀ 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]₀ under 25 mM K, all four heat components released under high-K perfusate were dependent on [Ca]₀. 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-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- or −P-to-P ratios. When P, H₃, H₂, and H₄ 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 verap-
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, H₄ was associated with mitochondrial activity. To test whether the magnitude of H₄ observed under high [K]₀ 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]₀ induced a caffeine-sensitive transitory increase in H₄ (23). It was suggested that the high [K]₀ 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]₀ perfusion induces an increase in the Na-K pump activity (23), a decrease in intracellular...
the relative increase in the exchange activity (in the Ca efflux mode) could explain exchanger would be expected. An increase in Na/Ca-Na and an activation of Ca removal by the Na/Ca exchanger would be less active. Such a hypothesis is the fact that H2 (which is a fraction of energy attributed to Ca removal (22)) grows more than H1 (which would represent, to some extent, the amount of Ca to be removed) compared with control conditions (22). In fact, because the energetic cost for Ca removal is one ATP per Ca removed via the Na/Ca 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]. 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 PT1-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] and [Ca] 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], 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] 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], and is coupled to mitochon-
Ca-dependent increase in mitochondrial activity. Furthermore, the time course of $H_4$ (developed for $>80 \text{s}$ 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 cytosolic Ca and P under 25 mM 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.

This work was supported by the University of Buenos Aires Grants OD-009 and OD-022 UBACYT República Argentina. M. T. Márquez and J. E. Ponce-Hornos are Established Investigators of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and A. E. Consolini is a Postdoctoral Fellow of CONICET. Address for reprint requests: J. E. Ponce-Hornos, Instituto de Investigaciones Cardiológicas, Fac. de Medicina, Univ. de Buenos Aires, Marcelo de Alvear 2270, 1122 Buenos Aires, Argentina. Received 28 January 1997; accepted in final form 10 June 1997.

REFERENCES