The heart extrasystole: an energetic approach

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Marengo, Fernando D., María T. Márquez, Patricia Bonazzola, and Jorge E. Ponce-Hornos. The heart extrasystole: an energetic approach. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H309–H316, 1999.—The consequences of an extrasystole (ES) on cardiac muscle's energetics and Ca2+ homeostasis were investigated in the beating heart. The fraction of heat release related to pressure development (pressure-dependent) and pressure-independent heat release were measured during isovolumic contractions in arterially perfused rat ventricle. The heat release by a contraction showed two pressure-independent components (H1 and H2) of short evolution and a pressure-dependent component (H3). The additional heat released by ES was decomposed into one pressure-independent (H1) and one pressure-dependent (H2) component with time courses similar to those of control components H2 and H3. ES also induced the potentiation of pressure development (P) and heat release during the postextrasystolic (PES) beat. The slope of the linear relationship between pressure-dependent heat and pressure maintenance was similar in control, ES, and PES contractions (0.08 ± 0.01, 0.10 ± 0.02, and 0.08 ± 0.01 mJ · g−1 · mmHg−1 · s−1, respectively). The potentiation of H2 (heat component related with Ca2+ removal processes) in PES was equal to H2 at 0.3, 0.5, 1, and 2 mM Ca2+, suggesting that the extra amount of Ca2+ mobilized during ES was recycled in PES. Pretreatment with 1 mM caffeine to deplete sarcoplasmic reticulum Ca2+ content inhibited both the mechanical and energetic potentiation of PES. However, the heat released and the pressure developed during ES were not changed by sarcoplasmic reticulum depletion. The results suggest that 1) the source of Ca2+ for ES would be entirely extracellular, 2) the Ca2+ stored during ES is accumulated in the sarcoplasmic reticulum, and 3) the Ca2+ stored by the sarcoplasmic reticulum during ES induces an increased contribution of this organelle during PES compared with the normal contraction.

cardiac muscle; calorimetry; sarcoplasmic reticulum; postextrasystolic beat

The relationship between developed pressure, Ca2+ homeostasis, and energy release during the extrasystole (ES) is an intriguing aspect in heart muscle physiology. Direct evidence of an increased Ca2+ mobilization during an ES was reported by Wier and Yue (29), who found under these circumstances an increase in the aequorin signal. Whether the additional Ca2+ is accumulated in the sarcoplasmic reticulum (SR) or it is removed to the extracellular matrix is an aspect of energetic relevance. This is because the energy expended for Ca2+ removal depends on the mechanisms involved. For instance, whereas the SR Ca2+ pump has a stoichiometry of two Ca2+ extruded per ATP hydrolyzed (27), the sarcolemmal Ca2+ pump has a stoichiometry of 1 Ca2+:1 ATP (5). If Ca2+ were to be removed by the Na+/Ca2+ exchanger, the cell would take up 3 Na+ per 1 Ca2+ removed. Because the Na+/K+ pump maintains a steady intracellular Na+ concentration by consuming 1 ATP per 3 Na+ transported, Ca2+ removal through the Na/Ca2+ exchanger would use 1 ATP per 1 Ca2+.

As it has been reported, an unambiguous measurement of various components of heart muscle energy utilization has been a desirable but difficult task to achieve (2, 9, 11, 25). The various approaches used have frequently required the independent, nonsimultaneous measurement of 1) basal metabolism, 2) tension-dependent energy released, and 3) tension-independent energy released (1, 2, 11, 12). From the difference between the total energy released and that elicited without force, the force-dependent energy release could be calculated (3, 9, 11, 13, 22). More recently, four components of heat released (H1, H2, H3, and H4) were simultaneously measured in a single contraction (21), and these energy fractions were related with processes known to accompany different periods of the twitch myogram. The most relevant aspect of these measurements is that the pressure-independent heat (we measured isovolumic pressure generation instead of force) was evaluated simultaneously with the pressure-dependent heat component (H3) in the presence of pressure development (P) (21). Pressure-independent heat 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 (H4) of long duration (21). Whereas H1 was tentatively associated (at least in part) with Ca2+ binding to troponin C (TnC), H2 was proposed to be mainly related to Ca2+ cycling processes (21). The H4 component showed a high dependence on mitochondrial respiration and Ca2+, and it is associated with a verapamil-sensitive process different from that related to force generation (6, 21). In the present study this approach was used to further investigate the energetic and mechanical consequences of an ES. The ES was induced 200 ms after the beginning of the regular beat, and the energetic and mechanical consequences of the postextrasystolic contraction (PES) were also studied. Additionally, the calcium sources associated with energy release and P for ES and PES were investigated. The results suggest an increased contribution of the SR for PES as compared with the normal contraction while the source of Ca2+ for ES would be entirely extracellular.

Methods

Biological preparation. Experiments were performed on 10 Wistar rats of either sex, weighing 250–300 g at the time of death. Each animal was heparinized (2,000 U) and anesthetized with a pentobarbital sodium overdose. The beating...
hearts were rapidly excised, and retrograde perfusion was initiated with a low-Ca\textsuperscript{2+} (0.5 mM) and 7 mM K\textsuperscript{+} perfusate at room temperature (20–24°C). The right atrium was carefully dissected from the heart, and a small cut in the septal wall, close to the aorta, was made to prevent spontaneous contractions. A latex balloon was placed into the left ventricle and the muscle mounted in the inner chamber of a calorimeter. The latex balloon was connected to a Statham P23Db pressure transducer so that pressure developed during isovolumic contractions could be measured. Optimal P was functionally established under stimulation (at ∼0.1 Hz) by gradually inflating the latex balloon (resting pressure was increased in steps of 10–15 mmHg) until stable twitch P showed no detectable increase at regular gain (188–375 mmHg full scale). All the results are quoted per gram of wet weight.

Solutions. The heart was perfused at a constant rate (5 ml/min) with a solution (control solution) containing (in mM) 1.3 MgCl\textsubscript{2}, 110 NaCl, 1.2 NaH\textsubscript{2}PO\textsubscript{4}, 0.5 CaCl\textsubscript{2}, 25 NaHCO\textsubscript{3}, 7 KCl, and 6.0 dextrose. The solutions were bubbled with 95% O\textsubscript{2}-5% CO\textsubscript{2} to achieve a pH of 7.3–7.4 (25°C). Under these experimental conditions, mechanical and energetic parameters remain reproducible for more than 6 h. In these experiments in which Ca\textsuperscript{2+} concentration in the perfusate ([Ca\textsuperscript{2+}]) was changed, no corrections for changes in osmolarity or in ionic strength were performed. The caffeine experiments were performed at 0.5 mM Ca\textsuperscript{2+} because at this Ca\textsuperscript{2+} concentration the muscles paced at 0.16 Hz remained without spontaneous contractions. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Mechanical and heat measurements. The technique for online measurement of heat production and mechanical activity of isolated heart muscle has been described previously in detail (8, 21, 24, 25). Briefly, the calorimeter was submerged in a constant-temperature bath. The temperature of the calorimeter bath (25°C) 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 (25). The minimum output of the thermosensitive units (two thermoelectric modules each containing 127 thermosensitive junctions) recorded in the present experiments was higher than 40 µV, whereas the electrical noise was between 1 and 2 µV at a maximum gain (1 µV/mm). With this method it was possible to record continuously and simultaneously resting pressure, P, pressure-time integral (PTI), perfusion pressure, total heat production (H\textsubscript{t}), and resting heat production (H\textsubscript{r}). 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. A muscle was accepted for study only if during the 60-min equilibration period a minimum of 105 mmHg steady pressure was developed at 0.16-Hz stimulation frequency at a resting pressure of 26 mmHg and remained quiescent in the absence of stimulation. Reproducible P values throughout the experiment were used as an indication of muscle stability, and this was checked several times (under control perfusate) during the experiment. The analysis of the energy components released in a contraction was performed as described elsewhere (21). Briefly, when power applied is interrupted before the integration time, the calorimetric output from zero time to its peak value can be fitted by the following equation (21)

$$H_t = H_o - 1 - A_o \cdot e^{-\beta \cdot t} - 8 \cdot \pi^2 \cdot \sum_{i=0}^{n} A_i \cdot e^{-\beta \cdot t} \cdot e^{-t}$$ (1)

where \(\mu\) is the cooling rate constant of the calorimeter, \(\beta\) is the diffusion delay constant, \(A_o = 1/(2 \cdot i + 1)^2 \cdot [1-(2 \cdot i + 1)^2 \cdot \mu - \tau], \alpha = (2 \cdot i + 1)^2 \cdot \mu, \tau = \) the rate constant of the declining fraction, t is time, \(H_o = (\mu \cdot 4 \cdot \pi^2 \cdot \beta^2 \cdot t^3) \cdot \tan[(\mu \cdot 4 \cdot \pi^2 \cdot \beta^2 \cdot t^3)]\), and \(H_o\) represents a fitted value of the power. The magnitudes of \(\beta\) and \(\mu\) were determined as described previously (21). The area under the whole calorimetric output curve represents the total energy released during the event. Therefore, from the fitted parameters, the total amount of energy released can be calculated, but no information in the time course of that release was presented. The various fractions of energy released during a contraction were fitted as a linear combination of components each described by Eq. 1 as shown elsewhere (21). Note that due to the delay associated to the calorimetric response, the time to peak of each component should not be considered as the time to peak of the process associated with it (for detailed explanation, see Ref. 21). Therefore, from this analysis, the amount of energy released by each component can be calculated, but no information is obtained on the time course of that release. From the differences between \(H_t\) and \(H_r\), divided by the frequency of stimulation, the active heat per beat was also calculated (21, 25).

The baseline used to measured the heat rate records of control and postextrasystolic beats was obtained from the calorimetric signal of the subsequent stimulus interval in which the stimulation was interrupted. In particular, the baselines for control beats (C) were obtained interrupting the stimulation during normal 0.16-Hz beating, and the baselines for PES were obtained interrupting the stimulation immediately after the application of an ES. Examples of baselines obtained with this methodology are illustrated in Fig. 5 (note dashed lines in Fig. 5, A and C).

The heat record associated with ES (Fig. 2B) was obtained from the difference between the heat record of the beat with ES (Fig. 1B) minus the heat record of the preceding C (Fig. 1A) (for further details see Ref. 21).

Statistical analysis. Data are presented as means ± SE, and statistical significance was set at P < 0.05. For paired comparisons, the paired t-test was used. Regression analysis was performed with the use of a nonlinear regression technique that uses the Marquardt algorithm running on an AT-386 compatible desk computer (21). The differences between the estimated and the hypothetical value (e.g., estimated slope against 1, correlation coefficients, and zero abscissa values against zero) as well as between two different correlation coefficients were analyzed as described elsewhere (7). Testing for symmetry and systematic deviations of the fitted curve from the data points was performed with the sign test (7). The comparison between fitted curves obtained with different number of terms for a given set of data points was performed with the Fisher test (7).

RESULTS

Constant [Ca\textsuperscript{2+}]o. We have recently shown that during steady stimulation of a heart muscle, an additional stimulus (200 ms after the regular one) releases an additional fraction of heat that can be decomposed into two components (21). To further investigate this phenomenon, muscles perfused with 0.5 mM Ca\textsuperscript{2+} control solution were paced at 0.16 Hz until steady mechanical and energetic parameters were observed. Under this condition, the mechanical and energetic effects of an extrasystolic stimulus (200 ms apart) on both the contraction that includes the extrasystolic stimulus and the PES were investigated. Figure 1 shows the
simultaneous myothermal and mechanical records from a C, a beat with ES, and a PES contraction. ES stimulus systematically induced an increase in the PTI (65.3 ± 5.3 vs. 86.3 ± 7.5 mmHg·s for C (n = 10) and contractions with ES (n = 10), respectively, P < 0.0005), with no changes in peak pressure (P) (197 ± 14 vs. 200 ± 14 mmHg for C and contractions with ES, respectively). On the other hand, for PES (n = 10) both P and PTI were potentiated compared with the C (+47 ± 11 mmHg and +15.8 ± 2.9 mmHg·s, P < 0.05). The heat released by each control contraction was fitted to a linear combination of three components of energy release, two of them independent of the development of pressure (H1 and H2) and a third one dependent on pressure generation (H3) (Fig. 2A). The average energy values measured for each component under control conditions were 1.9 ± 0.2, 4.3 ± 0.7, and 5.3 ± 0.7 mJ/g (n = 10) for H1, H2, and H3, respectively. Under this condition, a fourth component (also independent of pressure generation) of energy released (H4) was measured as the difference between active heat per beat and the sum of H1, H2, and H3 components, yielding an average value of 2.07 ± 0.67 mJ/g. When a second stimulus was applied 200 ms after the regular one (ES), additional heat was released above the control heat record (Fig. 2B). This additional fraction of heat could be fitted to only two components (H2 and H3) with
similar time courses to those observed for H2 and H3 during the control twitch (Fig. 2B). The average values for the energy released by these two components of additional heat were 0.7 ± 0.1 and 1.7 ± 0.3 mJ/g for H2 and H3, respectively (n = 10). It is of interest to note that, whereas H3 linearly correlated with the increase in PTI associated with the extrasystole (PTI_C - PTI_E) (r = 0.7483, for n = 10 at 0.5 mM Ca2+), the correlation of H2 with the same parameter was not significant (r = 0.05196, n = 10). These results also suggest a strong similarity in the origin of H3 and H2 in the extrasytotonic contraction with H3 and H2 in a regular contraction. PES contractions could also be fitted to a minimum of three components, H1, H2, and H3 (Fig. 2C), and their average energy values (2.6 ± 0.3, 5.0 ± 0.7, and 7.7 ± 1.3 mJ/g) were higher than the values for control contractions (+0.7 ± 0.2 mJ/g, +0.7 ± 0.2 mJ/g, and +2.48 ± 0.7 mJ/g) for H1, H2, and H3, respectively; n = 10). Variable [Ca2+]. To further investigate the origin of the calcium source associated with the energy released by ES, the dependence of mechanical and energetic parameters on [Ca2+], was studied. In each muscle, mechanical and energetic records from C, ES, and PES were obtained at four calcium concentrations (0.3, 0.5, 1, and 2 mM). The experiments were performed so that the initial Ca2+ concentration was always 0.5 mM Ca2+ but the changes in [Ca2+], were performed at random. In two experiments the increase in [Ca2+], to 2 mM induced spontaneous activity; therefore, those measurements (at 2 mM) were not considered. No measurements were considered for analysis until steady mechanical and energetic parameters for each calcium concentration were observed. The changes in additional heat components (H2 and H3) and in the PTI associated with ES (PTI_E - PTI_C) with [Ca2+], variation are summarized in Fig. 3. The three parameters (i.e., H2, H3, and PTI_E - PTI_C) increased with increasing [Ca2+]. Figure 3 also shows the [Ca2+], dependence of the postextrasystolic potentiation (i.e., the difference between PES and C) for H2, H3, and PTI. The energetic and mechanical dependencies on [Ca2+], associated on the potentiation of PES were different from those observed for the ES. At low [Ca2+], the potentiation of PTI and H3 (the energy component related to P) for PES was higher than the increase in PTI and H3 observed for the ES (Fig. 3). For PES, the potentiation in both PTI and H3 showed a peak at 0.5 mM [Ca2+], followed by a decrease at higher Ca2+ concentrations (Fig. 3). In fact, at 2 mM [Ca2+], these two parameters (H3 and PTI from PES - C; see Fig. 3) become significantly smaller than the corresponding parameters (PTI_E - PTI_C and H3) associated with ES (Fig. 3). When Ca2+ is increased to 2 mM Ca2+, the force generation was close to saturation with no margin for PES potentiation. It should be noted that the decline in PTI and H3 for PES - C at [Ca2+], > 0.5 mM was due to an increase with Ca2+ in heat components and P of control contractions.

The PES potentiation (i.e., the difference between PES and C) of H1 [an energy component that has been related to Ca2+ binding to TnC (21)] showed a dependence with [Ca2+], similar to that of PT1 and H3 (0.4 ± 0.2, 0.9 ± 0.3, 0.4 ± 0.2, and -0.2 ± 0.3 mJ/g for 0.3, 0.5, 1, and 2 mM Ca2+, respectively; data not shown in Fig. 3). On the other hand, PES potentiation of H2 showed a [Ca2+], dependency similar to that of the additional heat component H3 released during ES (Fig. 3). In fact, at the four [Ca2+], levels analyzed, no differences between H2 and H2 potentiation were found. Because the H2 component has been associated with Ca2+ cycling, these results suggest that the additional amount of Ca2+ mobilized during ES might be recycled during PES. Therefore, the PES potentiation could be explained if the additional calcium that entered during ES is stored in a cellular compartment and released during PES.

It has been shown that under control conditions, H3 linearly correlates with either P or pressure maintenance (21). Because for ES the P does not change but PT1 does, the increase in pressure-dependent energy expenditure should be related to the PT1 change. Consequently, the energy cost of the increment in PTI was studied to compare the economy of pressure maintenance between C, ES, and PES. As was already described for 0.5 mM Ca2+, when the data obtained at all four Ca2+ concentrations tested (0.3, 0.5, 1, and 2 mM Ca2+) was used, H3 linearly correlated with PTI (r = 0.8647). When H3 was plotted against the increase in PTI during ES, the regression parameters (0.10 ± 0.02 mJ/g mmHg, -1 mmHg, and 0.18 ± 0.54 mJ/g for the slope and zero axis intercept, respectively) were different from those obtained for H3 vs. PTI in C (0.08 ± 0.01 mJ/g mmHg, -1 mmHg, and 0.95 ± 1.71 mJ/g for the slope and zero axis intercept, respectively) (Fig. 4). Similar results were obtained when the same analysis was performed for PES. The regression of H3 vs. PTI for PES did not have an intercept different from zero (1.27 ± 0.63 mJ/g), and its slope (0.08 ± 0.01 mJ/g mmHg, -1 mmHg, and 0.95 ± 1.71 mJ/g for the slope and zero axis intercept, respectively) (Fig. 4). Therefore, these results indicate that the economy of pressure maintenance remained similar for the three types of contractions studied.

Caffeine effects. PES potentiation could be explained if the additional calcium that entered during ES is stored in a cellular compartment and released during PES. To test this hypothesis, five experiments were performed in which energetic and mechanical effects of 1 mM caffeine were investigated in muscles perfused under 0.5 mM Ca2+ conditions. This calcium concentration was chosen because 1) at this Ca2+ concentration the muscles paced at 0.16 Hz did not show spontaneous contractions and 2) as shown in Fig. 3, the largest potentiation of PES contractions was obtained under this [Ca2+]. For these experiments C, ES, and PES were recorded in the presence and in the absence of 1 mM caffeine. Caffeine was present for 30 min before any measurement was considered. In the presence of caffeine, P fell from 198 ± 29 to 100 ± 13 mmHg (n = 5, P < 0.01) and PTI was reduced from 60.2 ± 9.8 mmHg/g for control solution to 29.3 ± 4.2 mmHg/g (n = 5, P < 0.01). The maximal rate of relaxation was reduced from
614 ± 126 mmHg/s in control conditions to 328 ± 34 mmHg/s in the presence of caffeine. Also, the heat released by each contraction in the presence of caffeine (H1 + H2 + H3) decreased from 11.9 to 6.39 mJ/g (n = 5) due to the fall of all three components. Figure 5 shows an original record obtained in the presence of caffeine. The baseline shown in Fig. 5, A and C, was obtained by translating the calorimetric signal of a stimulus interval in which stimulation was interrupted. Note that PES potentiation disappeared from the mechanical and the energetic records. The averaged differences (evaluated as PES – C) for mechanical and energy parameters were not different from zero (2 ± 2 mmHg; −0.3 ± 0.8 mmHg·s; and 0.1 ± 0.08, 0.04 ± 0.08, and −0.3 ± 0.2 mJ/g for P, PTI, H1, H2, and H3, respectively). The ratios of H3 to P and H3 to PTI in the presence of caffeine increased (+0.49 ± 0.07 mJ·g⁻¹·mmHg⁻¹ and +0.021 ± 0.008 mJ·g⁻¹·mmHg⁻¹·s⁻¹, respectively, P < 0.05). This agrees with previous findings in which an increase in total active heat per unit of force development was found (3, 4).

Despite the fact that PTI was reduced by 50% in the presence of caffeine, the increase in PTI associated with ES was similar whether or not caffeine was present (17.4 ± 3.15 vs. 18.0 ± 2.8 mmHg·s, n = 5). It is of interest to note that in the presence of caffeine ES stimulus often induced an increase in P over the peak P developed during the C. When the difference of heat between the twin contractions in the presence of caffeine and the control contraction (also in the presence of caffeine) was analyzed, a minimum of 3 components were found. The first component, H1 (0.58 ± 0.18 mJ/g,
DISCUSSION

In the present work, resting heat values averaged 4.28 ± 1.14 mJ · s⁻¹ · g⁻¹. These values compare well with those previously reported by our laboratory and by other investigators (3, 10, 11, 23, 24). Under steady stimulation, the total activity-related heat liberation per beat averaged 14.5 ± 1.5 mJ/g, and that also agreed with values previously reported (14, 15, 21). It is of interest to note that the observed H1 and H2 values are within the range expected for the processes involved during the activation process. With the use of an enthalpy for the binding of Ca²⁺ to TnC between −32 and −26 kJ/mol Ca²⁺ (16, 19), the H1 component found in the present work (1.9 mJ/g) would represent between 59 and 73 nmol Ca²⁺/g. If all this Ca²⁺ is to be removed by the sarcolemmal or sarcorticular pumping systems (1 and 2 Ca²⁺ removed per 1 ATP, respectively), the heat expected (using 44 kJ/mol creatine phosphate and using an intermediate Ca²⁺-to-ATP ratio of 1.5) from these processes would be ~1.7–2.1 mJ/g. Although these values are lower than the H2 measured (4.3 mJ/g), it is reasonable to expect that the total Ca²⁺ released would be higher than that actually bound to TnC. Consequently, the heat output for Ca²⁺ removal should be higher than that expected for the removal of the Ca²⁺ bound to TnC. In addition, this difference leaves energy to be related to other ionic movements such as Na⁺ and K⁺ (21).

The energetic and mechanistic parameters measured for ES and PES indicated that the economy of pressure maintenance remained similar for the three types of contractions studied. Although the energy associated to ES was smaller than that obtained for the C, the relationship between H1 and the associated increase in PTI was similar to that found in control contractions for H3 versus PTI. Because a similar H3-to-PTI ratio was found for PES, it can be concluded that the economy of pressure maintenance has not been affected by the extra stimulus. It is of interest to note that, during ES, if H2 (0.7 mJ/g) is fully attributed to SR Ca²⁺ removal, it would represent ~32 nmol of Ca²⁺ associated with the second stimulus (using now a Ca²⁺-to-ATP ratio of 2). The increase in H1 component observed for PES was ~0.7 mJ/g, which is the heat expected for the binding of 22–27 nmol Ca²⁺ to TnC. This agrees with a process in which most of the Ca²⁺ associated with the second stimulus (and captured by the SR) would have been released during the PES and used to generate force.

The results obtained in the presence of caffeine support the idea that the mechanical event related to ES depends on an extracellular Ca²⁺ source. Although the P decreased to 50% in the presence of 1 mM caffeine, the increase in PTI induced by ES was similar to that observed for control conditions. Also, caffeine failed to affect the second (Ca²⁺ dependent) component of energy release associated with ES. In this regard, an additional Ca²⁺ influx through sarcolemmal Ca²⁺ channels has been suggested to occur during ES (18). As it happens with H2 component [an energy component that has been related to pumping Ca²⁺ (21)], the H2 component released during ES is also dependent on [Ca²⁺]c. The saturation of H2 component and PTI associated with ES at Ca²⁺ concentrations higher than 1 mM suggests that the amount of Ca²⁺ cycled during this event is saturated (Fig. 3). These results could be explained by saturation at the level of Ca²⁺ influx. This interpretation is in agreement with Wang et al. (28), who found that, in the absence of EGTA in the patch pipette (to mimic physiological conditions), the area under Ca²⁺ current saturates at ~2 mM Ca²⁺.

The absence of a postextrasystolic pressure enhancement with the caffeine pretreatment suggests that all the Ca²⁺ that entered the cell during ES was removed before the next twitch. In the presence of caffeine the
capacity of the SR to retain Ca\(^{2+}\) is reduced. Consequently, the "extra" Ca\(^{2+}\) that entered during ES should have been removed to a site different from the SR and should not be available for the next twitch. It can be hypothesized that under control conditions the additional Ca\(^{2+}\) that entered the cell during ES could be accumulated by the SR and released by the next contraction. Caffeine impairs the accumulation of Ca\(^{2+}\) by the SR and consequently prevents PES pressure enhancement. On the other hand, H2 potentiation (PES – C) was similar to the H\(_2\) component in the entire range of [Ca\(^{2+}\)] tested. This suggests that the whole amount of Ca\(^{2+}\) accumulated by the SR and released by the next contraction. Caffeine impairs the accumulation of Ca\(^{2+}\) under the control contraction yielding a smaller difference with ES stimulus was released to the cytosol during PES. Therefore, the saturation of H2 potentiation during PES would be an indirect consequence of Ca\(^{2+}\) influx saturation during ES. On the other hand, even though H2 potentiation either increased or remained unchanged with the increase in extracellular Ca\(^{2+}\) (suggesting a higher amount of Ca\(^{2+}\) cycling), the potentiation observed for PTI, H3, and H1 became smaller as extracellular Ca\(^{2+}\) increased. This could be due to a progressive saturation of the myofilaments under the control contraction yielding a smaller difference with the PES contraction.

It is of interest to note that the ES in the presence of caffeine induced an increase in peak P that was absent under control perfusion. This increased P was accompanied by an early component of heat similar to the H1 found in the regular contraction and supports the origin of H1 as related to binding Ca\(^{2+}\) to TnC (21). The results seem to indicate that the additional Ca\(^{2+}\) entry induced by ES in control conditions fails to increase the Ca\(^{2+}\) binding to TnC (note that there is no increase in P, but the duration of the twitch is prolonged). In contrast, in the presence of caffeine, a similar additional Ca\(^{2+}\) entry induces a clear increase in P, probably because the rate of Ca\(^{2+}\) removal from myofilaments is decreased (the maximal rate of relaxation was markedly reduced by caffeine). In consequence, the additional Ca\(^{2+}\) entry in the presence of caffeine should produce a net increase in Ca\(^{2+}\) binding to TnC. This additional Ca\(^{2+}\) binding would then release the H1 described under this condition.

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