A unique micromechanocalorimeter for simultaneous measurement of heat rate and force production of cardiac trabeculae carneae

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Han J-C, Taberner AJ, Kirton RS, Nielsen PM, Smith NP, Loiselle DS. A unique micromechanocalorimeter for simultaneous measurement of heat rate and force production of cardiac trabeculae carneae. *J Appl Physiol* 107: 946–951, 2009. First published July 9, 2009; doi:10.1152/japplphysiol.00549.2009.—To study cardiac muscle energetics quantitatively, it is of paramount importance to measure, simultaneously, mechanical and thermal performance. Ideally, this should be achieved under conditions that minimize the risk of tissue anoxia, especially under high rates of energy expenditure. In vitro, this consideration necessitates the use of preparations of small radial dimensions. To that end, we have constructed a unique micromechanocalorimeter, consisting of an open-ended flow-through microcalorimeter, a force transducer, and a pair of muscle-length actuators. The device enables the metabolic and mechanical performance of cardiac trabeculae carneae to be investigated for prolonged periods in a continuously replenished oxygen- and nutrient-rich environment.

muscle energetics; microcalorimetry; heat-stress relation; activation heat

CARDIAC MUSCLE IS A THERMODYNAMIC machine. It is capable of directly converting a fraction of biochemical enthalpy into external work, with the remainder degraded to heat. During fixed-end (i.e., quasi-isometric) contractions, in which the external work is zero, the rate of heat production thus provides an index of the rate of metabolic enthalpy expenditure. Because mechanics and metabolism are tightly coupled in cardiac muscle, it is advantageous to measure them simultaneously (i.e., in the same preparation), thereby obviating the need for experiments conducted in parallel.

In cardiac muscle, enthalpy expenditure is funded almost exclusively by mitochondrial oxidative phosphorylation. Thus, for studies of cardiac muscle function in vitro, it is important to choose preparations of sufficiently small radial dimensions so that their enthalpy expenditure is not compromised by anoxia. ter Keurs et al. (28) largely obviated this problem by use of cardiac trabeculae carneae, which typically have radial dimensions of ~100 μm. Daut and Elzinga (5, 6) developed a flow-through microcalorimeter suitable for measuring the rate of heat production of such preparations. Using the device, they were able to report the rates of heat production of continuously superfused cardiac trabeculae with submicrowatt resolution. Despite this remarkable technological achievement, simultaneous force measurements remained wanting, despite numer-

ous attempts at implementation. We can now report success in this endeavor.

We have constructed a unique flow-through micromechanocalorimeter, the microcalorimetric moiety of which is based on the Daut-Elzinga principle. That is, we measure the increment of temperature of superfusate, via noncontact thermopile sensors, as it flows past a respiring trabecula. Our breakthrough to making simultaneous mechanical measurements followed our decision to adopt an open-ended design for the measurement chamber of the microcalorimeter. Open-endedness allows insertion of a tubular quartz arm into either end. The upstream arm connects to a pair of linear actuators, arranged in series. This arrangement allows both macroscopic change of muscle length (for determination of the heat-length relationship of the trabecula) and microscopic, variable-frequency perturbation of muscle length (for determination of cross-bridge stiffness). The downstream quartz arm connects to an off-the-shelf force transducer. The trabecula is connected between the two arms. This arrangement enables the first simultaneous measurements of the mechanical performance and the accompanying rate of heat production of continuously superfused cardiac trabeculae carneae.

METHODS

The Micromechanocalorimeter

Design. A diagram of the micromechanocalorimeter is presented in Fig. 1. Its microcalorimetric component has been described previously (27). Briefly, it comprises an open-ended measurement chamber, consisting of a borosilicate glass tube of square cross-section (1 mm inner width, 7 mm long) glued into a gold-plated brass housing. Inserted into both ends of this glass tube are tubular quartz arms (700 μm outer and 500 μm inner diameters) to which are glued J-shaped platinum hooks for securely attaching the muscle. The upstream quartz arm is attached to a Queensgate piezoelectric actuator (Queensgate Technologies), which, in turn, is attached to a position actuator (M-227.25 DC-Mike, Physik Instrumente). The downstream quartz arm is attached to a silicon-beam force transducer (KX801 Micro Force Sensor, Kronex Technologies). Thermopile sensors (each consisting of 100 Bi-Sb thermocouples deposited on a Si_3N_4 membrane) are mounted 4 mm apart on the outer surfaces (bottom and both sides) of the glass tube. The trabecula is located in the center of the measurement chamber, midway between the thermopile arrays. The measurement chamber is continuous with an open-topped, open-ended muscle-mounting chamber downstream. The entire device is placed within an optically isolated, thermally insulated enclosure on a vibration-free optical breadboard.

Control. At its maximum displacement of ±5 μm, the frequency response of the Queensgate piezoelectric actuator is flat to 100 Hz. It is driven from a data-acquisition card (PCI-6221, National Instruments), which simultaneously monitors its position as well as acquiring the voltage signal from the force transducer. The Physik Instrumente
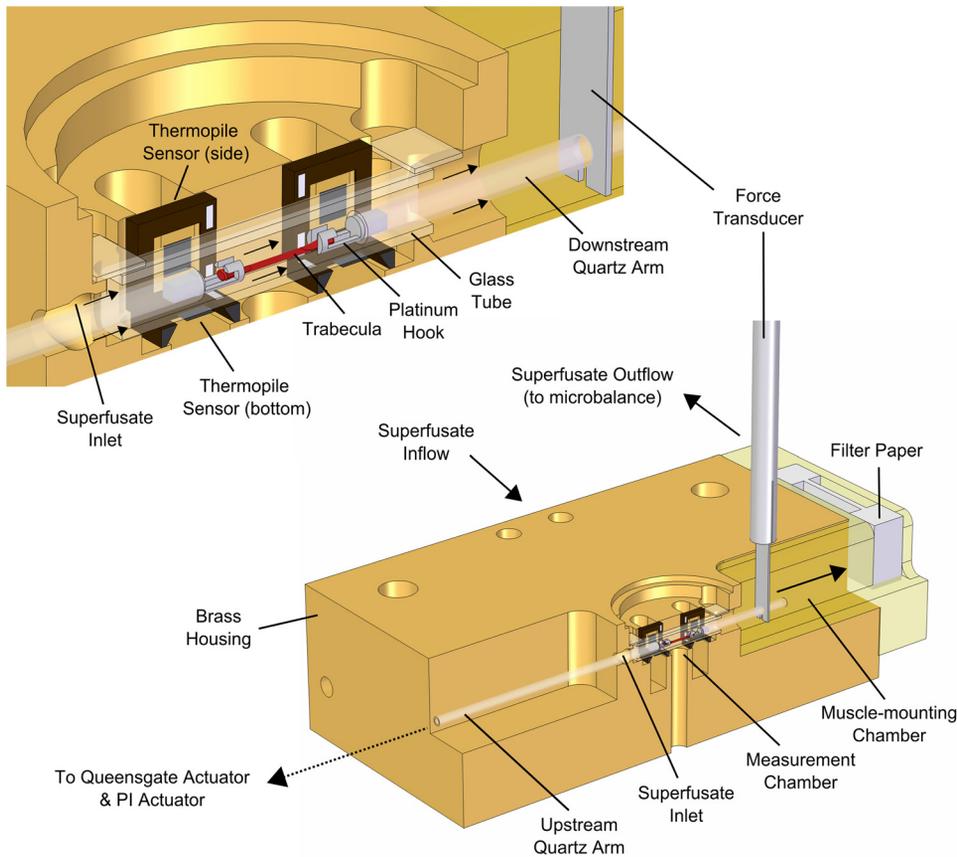
Fig. 1. Cut-away views of the micromechanocalorimeter. *Top*: the microcalorimeter consists of a borosilicate glass tube and 3 externally mounted thermopile arrays located upstream and downstream. Superfusate enters the measurement chamber via an upstream inlet (which penetrates the brass block), flows over the trabecula (which is mounted between a pair of platinum hooks), and is collected downstream (the arrows indicate the direction of flow). Thermopile arrays sense the temperature of superfusate upstream and downstream of the centrally located trabecula. The rate of heat production of the preparation is proportional to the product of the difference of temperature and the rate of flow of superfusate. *Bottom*: expanded view of the micromechanocalorimeter. Each platinum hook is glued to a quartz arm. The upstream arm connects to the Queensgate actuator (for determination of stiffness) and, in turn, to the Physik Instrumente actuator (for change of position or length of the preparation). The downstream arm connects to the force transducer, which also lies outside the measurement chamber. Filter paper at the superfusate outlet prevents micromeniscus disturbances to flow. The mechanical components at each end are mounted on 3-axis micromanipulators (not shown).

actuator has a maximum travel range of 25 mm and a maximum velocity of 1 mm/s. It is controlled by a motor-controller card (PCI-7344, National Instruments). The voltage signal from each thermopile array is measured using a nanovoltmeter (2182, Keithley) and transferred to a computer via a GPIB interface. Data are acquired using LabVIEW 8.5 and are recorded using LabVIEW SignalExpress 2.5 (both from National Instruments).

Superfusate. Superfusate flows into the measurement chamber via an inlet near the open upstream end. Surface tension prevents spillage. After it passes over the trabecula, exudate is collected in a microbalance (Scout Pro, Ohaus, Pine Brook, NJ) where its mass is sampled at 7.14 Hz. A straight line is fitted to the most recent 1,000 samples; its gradient estimates the instantaneous rate of flow. Pulsatile changes of meniscus shape, which are a potential source of noise on the force transducer signal, are avoided by the presence of filter paper at the downstream outlet. Exudate traverses the filter paper by capillary action and is continuously aspirated from the distal end by a vacuum pump. Hence, a steady flow of superfusate (nominally 1 µl/s) is achieved by the combination of upstream gravity-feed coupled with downstream nonpulsatile fluid removal.

Calibration. The Daut and Elzinga microcalorimeter (5, 6) was calibrated using a "point-source" thermistor located midway between the upstream and downstream thermocouples. To avoid the possibility that calibration may be misinterpreted by the resulting establishment of steep thermal gradients, we have chosen to use a "distributed source." To that end, we attached a 1-mm-long thin-film resistor to the upstream quartz arm and advanced it to lie midway between the arrays of upstream and downstream thermopiles. Rectangular pulses of electrical power (1 mW; on and off durations of 30 s, respectively) were liberated repetitively for 1 h. Over the 1-h period, the flow rate was calculated to be 0.9957 ± 0.0362 µl/s (mean $\pm$ SD). The sensitivity of the microcalorimeter is given by ratio of the differential voltage output of the thermopile arrays to the electrical power input

from the resistor. Its sensitivity is 1.6050 ± 0.0028 V/W (mean $\pm$ SD) at the above flow rate.

The force transducer was calibrated by suspending known masses from its beam. It has a sensitivity of 875.00 ± 0.29 V/N (mean $\pm$ SD). The frequency response of the complete mechanical system is flat, within 3 dB, to 100 Hz; its first resonant frequency occurs at 1.3 kHz.

Experimental Protocols

To demonstrate the range of capabilities of the micromechanocalorimeter, we subjected right ventricular trabeculae from adult Wistar rats to several chronotropic, ionic, and pharmacological interventions. All experiments were conducted according to protocols approved by The University of Auckland Animal Ethics Committee.

Muscle preparation. Rats were deeply anesthetized using isoflurane prior to decapitation, thoracotomy, and cardiectomy. Geometrically uniform trabeculae were dissected from the right ventricular free walls in a bath containing dissection solution of the following composition (mM): 130 NaCl, 6 KCl, 1 $MgCl_2$, 0.5 NaH_2PO_4, 0.3 $CaCl_2$, 10 HEPES, 10 glucose, and 20 2,3-butanedione monoxime, at pH 7.4 (adjusted with 1 M Tris), which was continuously bubbled with 100% O_2. The preparation was transferred to the mounting chamber where it was gently manipulated onto the hooks. A small block of tissue at either end of the trabecula, extirpated from the ventricular free wall, ensured secure attachment to the hooks. The trabecula was then translated into the measurement chamber. The experimental superfusate was the same as the dissection solution except for the absence of 2,3-butanedione monoxime and an increase of calcium concentration. Both dissection and experiments were performed at room temperature (20–22°C).

Electrical stimulation. Trabeculae were field stimulated at 0.2 Hz using 3-V, 5-ms pulses generated by the PCI-6221 card and delivered

via a platinum plate electrode located in the muscle-mounting chamber. The preparation was gradually stretched to the length (L_o) at which active force generation was maximal. Stimulus heat (which averaged 0.31 μW/Hz, at a flow rate of 1 μl/s) was quantified at the end of an experiment by running the stimulator using the same stimulus parameters but in the absence of the trabecula. The rate of muscle heat production was corrected retrospectively. Viscous heating (20) was assumed to be negligible.

Normalization of data. By approximating the geometry of each trabecula as a circular cylinder, the rate of heat production of the trabecula was normalized to its wet volume (reported in units of mW/cm^3). Force production was normalized to muscle cross-sectional area (and expressed in units of kPa).

Statistical Analysis

The heat-stress data measured in a single trabecula subjected to two different concentrations of extracellular calcium were fitted by quadratic regression using the Statistica software package. The difference in intercepts between the two relations was examined for statistical significance (at the 95% level of confidence) using the generalized nonlinear models module.

RESULTS

Effects of Stimulus Frequency, Extracellular Calcium Concentration, and Muscle Length

Figure 2 shows the effects of varying stimulus frequency, extracellular calcium ion concentration ($[Ca^{2+}]_o$), and muscle length on force production and heat rate measured

simultaneously. The top two traces show the signals of the upstream and downstream thermopile arrays. The third trace shows the rate of heat production by the trabecula, and the bottom trace shows its normalized force production. Figure 2 illustrates several notable features. *1*) The heat produced by individual twitches at 0.2 Hz is discernible. *2*) Negative treppe, characteristic of rat myocardium at room temperature, is evident in the force traces. *3*) Elevation of $[Ca^{2+}]_o$ from 1 to 2 mM increases the steady-state twitch force production. *4*) Reduction of muscle length below L_o, at a fixed value of $[Ca^{2+}]_o$, is reflected in the diminution of baseline force, twitch force production, and the rate of heat production.

Sinusoidal Perturbations of Muscle Length

As shown in Fig. 3*A*, we exploited the ability of caffeine (10 mM), in the presence of elevated $[Ca^{2+}]_o$ (5 mM), to induce a tetanus in response to high stimulus frequency (10 Hz). It may be noted that the twitch-to-tetanus ratio is near unity, as has previously been reported for rat left ventricular papillary muscles undergoing caffeine-induced contractures (7). When tetanic force and heat rate had stabilized, we imposed small-amplitude (0.124% L_o) sinusoidal length perturbations, at five perturbation frequencies, via the piezoelectric actuator. The region of interest in the force trace is amplified in Fig. 3*A*, *inset*. These data, from which it is evident that stiffness increases with perturbation frequency,
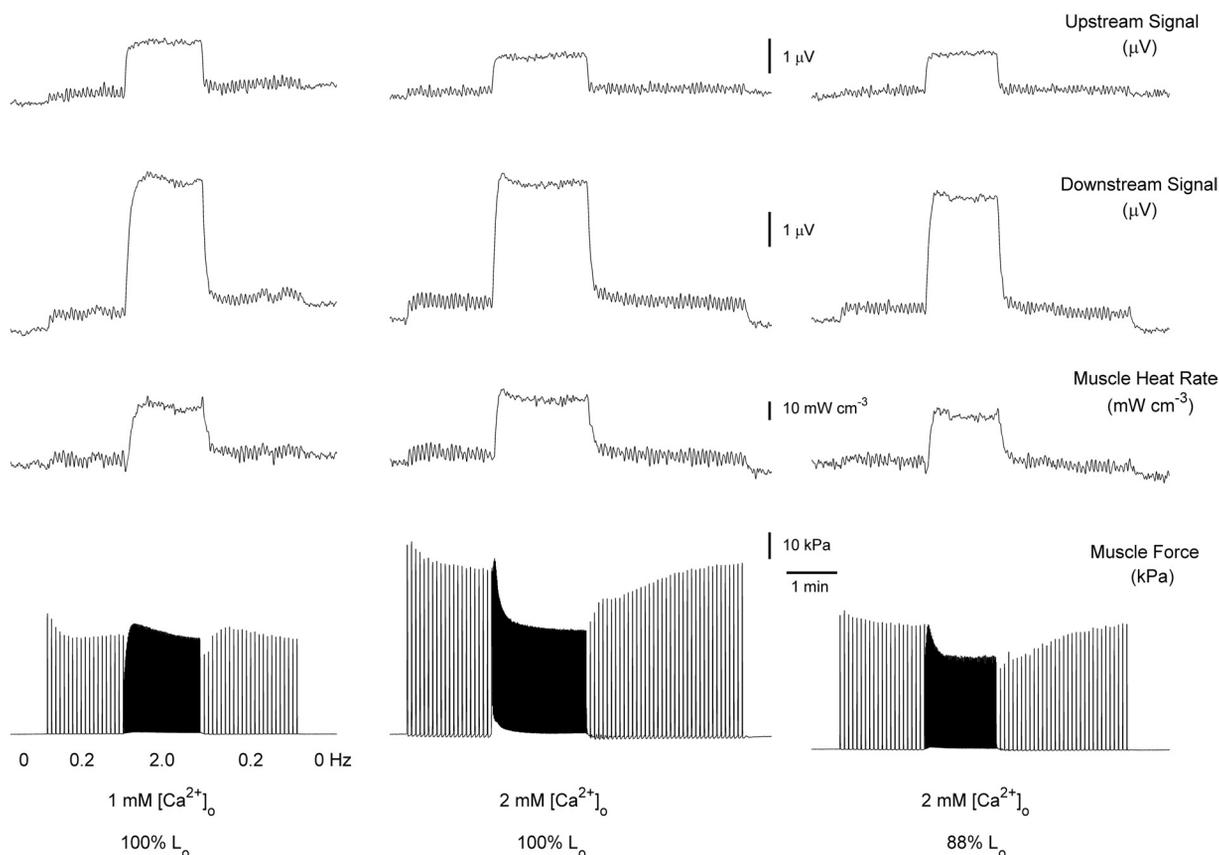

Fig. 2. Typical records of upstream and downstream thermopile signals, the resulting calculated rate of heat production, and the corresponding twitch force at different values of stimulus frequency, extracellular calcium concentration ($[Ca^{2+}]_o$), and muscle length (%L_o). The rate of muscle heat liberation is proportional to the difference between upstream and downstream thermopile signals and has been corrected for the stimulus heat. The trabecula was 150 μm in diameter and 1.7 mm in length.
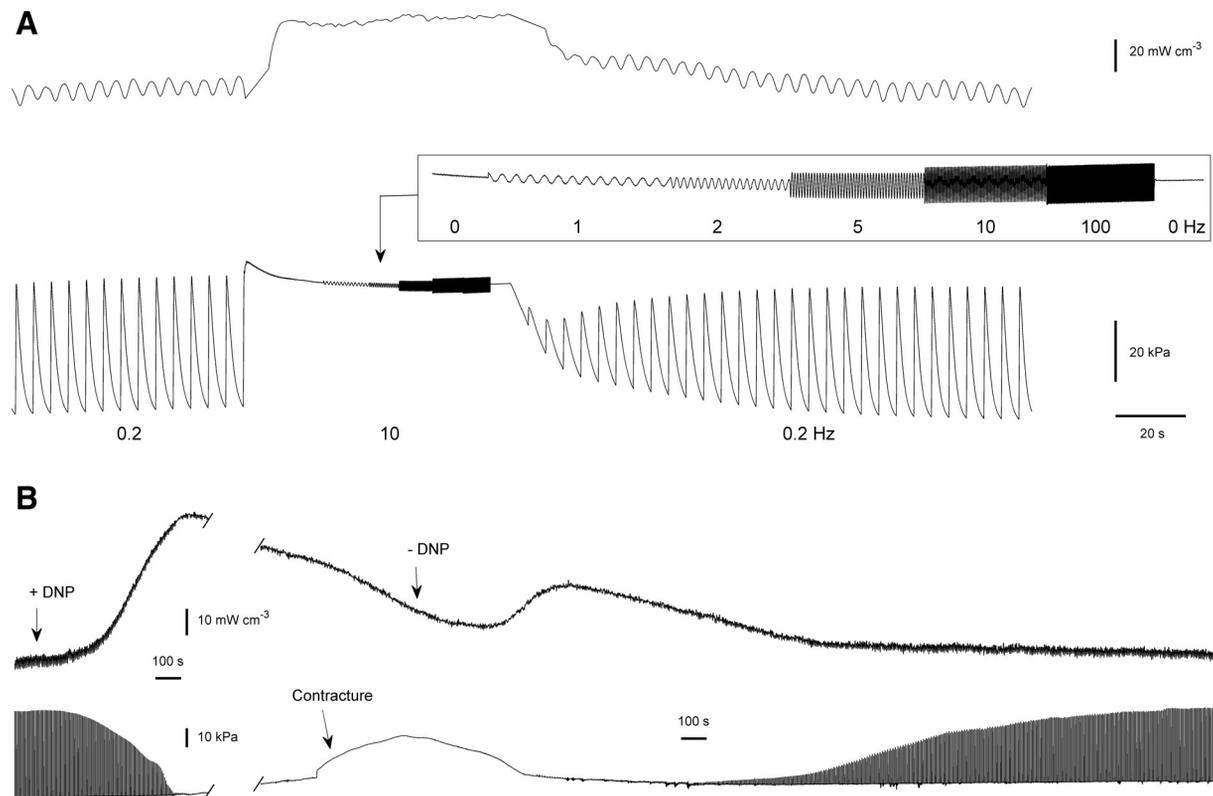
Fig. 3. Outputs of the micromechanocalorimeter in response to 2 pharmacological interventions. *A*: in the presence of 10 mM caffeine and 5 mM $[Ca^{2+}]_o$, 10-Hz electrical stimulation elicited a fully fused tetanic contraction (*bottom trace*) and resulted in an increased rate of heat production (*top trace*). During the tetanus, muscle length was perturbed by 0.124% L_o at 5 perturbation frequencies: 1, 2, 5, 10, and 100 Hz. The trabecula was 180 μm in diameter and 3.1 mm in length. *B*: application of 2,4-dinitrophenol (DNP; 0.1 mM) greatly increased the rate of heat production (*top trace*), while leading to the abolition of twitch force (*bottom trace*) in response to 0.2-Hz stimulation, followed by induction of a contracture, from which the preparation recovered subsequent to washing out of DNP. The trabecula was 200 μm in diameter and 4.0 mm in length.

provide the foundation for constructing the stiffness spectrum of the trabecula (11, 15).

Uncoupling of Oxidative Phosphorylation Using 2,4-Dinitrophenol

An independent intervention (Fig. 3*B*) focused on generating a sustained high rate of heat production. This was achieved by using 2,4-dinitrophenol (DNP), an uncoupler of mitochondrial oxidative phosphorylation. As can be seen in Fig. 3*B*, after administration of DNP (*left arrow*), the rate of heat production was greatly enhanced. Simultaneously, twitch force diminished to zero, presumably reflecting the progressive depletion of phosphocreatine (PCr) and accumulation of inorganic phosphate with its inhibitory effect on cross-bridge cycling (12) and sarcoplasmic reticular calcium content (25). Subsequently (after the 2-min break in the record), the preparation developed a contracture, presumably in response to depletion of ATP. Washout of DNP (Fig. 3*B*, *right arrow*) prompted relaxation from the contracture as oxidative phosphorylation recommenced. The combination of the restoration of ATP and PCr, coupled with the first reappearance of twitches, elicited a secondary burst of heat, which then returned to a level only slightly in excess of its rate of production before the intervention. Despite this prolonged metabolic insult, twitch force recovered to just over 90% of its preintervention value. Passive force remained slightly elevated.

Heat-Stress Relations of a Single Trabecula

Whereas Figs. 2 and 3 present examples of raw data, Fig. 4 provides the first demonstration of the heat-stress relation of a cardiac trabecula carnea. Muscle stress (force per cross-sectional area) development was varied by varying *1*) $[Ca^{2+}]_o$, *2*) muscle length, and *3*) stimulation frequency. We deliberately chose values of $[Ca^{2+}]_o$ (1 and 2 mM) only slightly above and slightly below the normal value of ionized calcium (1.5 mM) of rat plasma, thereby challenging the device to discriminate small differences in heat output. The success of this challenge is reflected in the statistically significant difference ($P < 0.05$) of intercepts between the two quadratic relations: 2.51 and 3.86 mJ/cm^3 per twitch at 1 and 2 mM $[Ca^{2+}]_o$, respectively. The intercepts on the ordinate correspond to the absence of macroscopic force development. Hence, their values provide estimates of the $[Ca^{2+}]_o$ dependence of the cost of calcium uptake from the myoplasm by the sarcoplasmic reticulum. Given the association with calcium activation of contraction, this thermal component is known as the "activation heat."

The magnitude of activation heat has previously been shown to vary directly with $[Ca^{2+}]_o$ (8) and inversely with temperature (17), reflecting the high temperature dependence of the sarcoplasmic reticulum Ca^{2+}-ATPase (26). In flat-bed thermopile studies at 27°C, in which rat left ventricular papillary
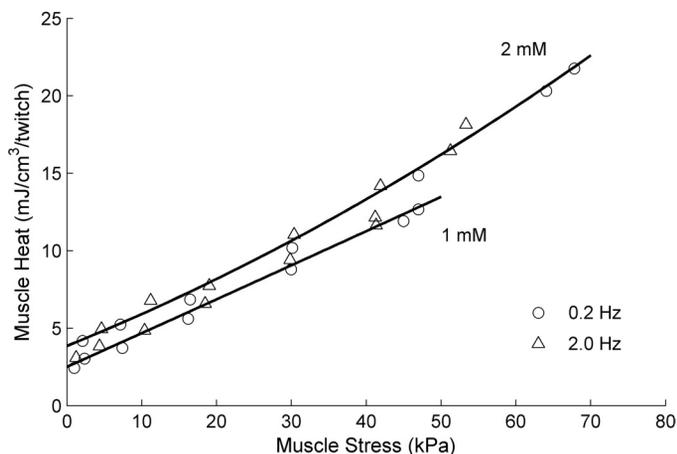
Fig. 4. The heat-stress relation of a single right ventricular trabecula. Stress was varied by varying $[Ca^{2+}]_o$ (1 or 2 mM), stimulus frequency (0.2 or 2.0 Hz), and muscle length from L_o (length at which active force development was maximal) to the length at which active force production was negligible. Note that heat (J) is normalized per cm^3 of tissue and expressed "per twitch"; stress (kPa) is calculated as force per cross-sectional area. The heat (H)-stress (S) relations were fitted using 2nd-order polynomials with the following results: $H_{1\ mM} = 2.51 + 0.22S + 4.92 \times 10^{-5} S^2$, $s_{y.x} = 0.37$, $r^2 = 0.992$ and $H_{2\ mM} = 3.86 + 0.20 S + 1.04 \times 10^{-3} S^2$, $s_{y.x} = 0.46$, $r^2 = 0.995$, where $s_{y.x}$ is the standard error of regression and r^2 denotes the proportion of total variance explained by regression. The trabecula was 220 μm in diameter and 2.3 mm in length.

muscles were stimulated at 1/6 Hz in the presence of 2.5 mM $[Ca^{2+}]_o$, mean values of 1.6 to 1.7 mJ/g were obtained (17, 18, 21). At 20°C, under the same stimulation protocol (17), the mean value increased to 2.8 mJ/g. In those studies, the cross-sectional area of the papillary muscles varied from 0.7 to 1.0 mm^2.

Kiriazis and Gibbs (13, 14) perfected the technique of splitting papillary muscles longitudinally, thereby roughly halving the cross-sectional area. Using the same preshortening procedure at 21°C, mean values of 1.7–2.6 mJ/g were found for these split muscles. In comparison, we found values for the single trabecula shown in Fig. 4 of 2.51 and 3.86 mJ/g at 20°C in 1 and 2 mM $[Ca^{2+}]_o$, respectively (where the units mJ/g and mJ/cm^3 are equivalent under the assumption of unit tissue density). These values are somewhat higher than those gleaned from the literature, possibly reflecting the combined advantages of continuous superfusion and a more favorable radial dimension for the diffusive supply of oxygen.

DISCUSSION

We have devised and constructed a flow-through micromechanocalorimeter for measuring, simultaneously, the rate of heat production and force production of cardiac trabeculae carneae. The device offers a number of advantages over its antecedents.

Advantages

Simultaneous measurement. The paramount advantage of our micromechanocalorimeter, vis-à-vis the original Daut and Elzinga microcalorimeter (5, 6, 16, 24), is of course the ability to make mechanical measurements simultaneously with the measurement of heat production. Previously, measurements of heat and force production of isolated trabeculae had to be performed in parallel (19, 20). Although "parallel experiments"

could yield data comparable to those shown in Figs. 2 and 3 (albeit using different preparations), they could not be used to produce heat-stress relations as depicted in Fig. 4.

Continuous superfusion. Unlike the myothermic technique (in which the muscle preparation is in contact with a flat-bed thermopile from which the static bathing solution has been withdrawn to measure heat production), the trabecula is continuously irrigated in a flowing stream of superfusate, thereby providing it with a steady source of nutrients while simultaneously removing its metabolic waste products.

Avoidance of heat of condensation. When a flat-bed thermopile is used, during the intermittent periods of measurement, the muscle preparation is in contact with a gas that is saturated with water vapor. Contraction increases the osmolarity of the muscle, as a consequence of the immediate breakdown of monomolecular PCr to creatine and inorganic phosphate. In consequence, water vapor condenses on the muscle, thereby liberating heat and raising the temperature of the muscle (10, 22). This problem is greater when the surface area-to-volume ratio of the preparation is larger. It would thus be an issue if heat production of trabeculae were to be measured using flat-bed thermopiles. The problem is entirely obviated in the flow-through microcalorimeter, independent of the size of the muscle.

Minute preparations. Preparations can be utilized that are comparable in diameter to amphibian single skeletal muscle cells (2–4) and are much smaller than papillary muscles, even those arising from the mouse right ventricle. Thus concerns regarding insufficient oxygenation by diffusion in vitro (1) are largely obviated.

Stiff muscle attachment system. The use of quartz connecting arms and platinum hooks (Fig. 1) provides a stiff mechanical arrangement for tethering the trabecula. It is this feature that allows us to interrogate cross-bridge stiffness using (sinusoidal) perturbations of muscle length (Fig. 3*A*).

Disadvantage

The sole disadvantage of the heat measuring component of the system, vis-à-vis that of classical flat-bed thermopiles designed for cardiac muscle preparations (9, 23), is its lower frequency response. This is an obligatory corollary of its flow-through advantage. In consequence, it is not possible to resolve the heat production of individual twitches.

As with any multi-cell cardiac preparation, sarcomere length is heterogeneous throughout the volume of a trabecula. (Laser diffraction techniques provide only an average value over the illuminated region.) Hence, during a fixed-end (quasi-isometric) contraction, some cells will contract concentrically, whereas others will contract eccentrically. The thermal consequences of these different modes of contraction at the microscopic level become indistinguishable at the macroscopic level.

Conclusion

The micromechanocalorimeter exhibits sufficient sensitivity to measure the rate of heat production of isolated cardiac trabeculae, even under low rates of stimulation and at room temperature. Coupled with the ability to vary muscle length and to measure muscle force, it provides an innovative device with which to extend our understanding of the energetics of cardiac muscle.

To the best of our knowledge, our micromechanocalorimeter is unique.

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