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ACTIVATION HEAT IN RABBIT CARDIAC MUSCLE

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SUMMARY

1. Activation heat was estimated myothermically in right ventricular papillary muscles of rabbits using several different methods.

2. Gradual pre-shortening of muscles to a length (l_{min}) where no active force development took place upon stimulation led to relatively low estimates of activation heat ($1{\cdot}59 \pm 0{\cdot}26$–$2{\cdot}06 \pm 0{\cdot}57$ mJ g^{-1} blotted wet weight, mean ± S.E.M., $n = 10$).

3. Quick releases applied during the latency period, before force development, from l_{max} to various muscle lengths allowed a heat–stress relation to be established. The zero-stress intercept of this relation estimated the activation heat to be $3{\cdot}27 \pm 0{\cdot}40$ mJ g^{-1}; this was close to the experimentally measured value of $3{\cdot}46 \pm 0{\cdot}39$ mJ g^{-1} (mean ± S.E.M., $n = 23$) found by quick release from l_{max} to l_{min}.

4. The magnitude of the activation heat measured by the quick-release technique is dependent upon the extracellular Ca^{2+} concentration and there is good correlation between activation heat magnitude and peak developed stress.

5. In agreement with expectations based on the aequorin data of Allen & Kurihara (1982) a prolonged period of time spent at a short length is shown to depress the subsequently determined activation heat.

6. Hyperosmotic solutions ($2{\cdot}5 \times$ normal) only abolished active stress development at low stimulus rates (0·2 Hz) and the activation heat measured at l_{max} under these conditions was $2{\cdot}03 \pm 0{\cdot}12$ mJ g^{-1} (mean ± S.E.M., $n = 6$). This value was significantly lower than the latency release estimate of activation heat in the same preparations ($2{\cdot}93 \pm 0{\cdot}39$ mJ g^{-1}).

7. The latency release method of estimating activation heat results in activation heat values that account for approximately 30% of total active energy flux per contraction; a fraction comparable to that found in skeletal muscle. Calculations based on the data suggest that, under our experimental conditions, total Ca^{2+} release per beat lies between 50 and 100 nmol g^{-1} wet weight which would produce less than half-maximal myofibrillar ATPase activity when allowance is made for the passive Ca^{2+}-buffering capacity of the myocardial cell.

INTRODUCTION

In all muscle tissue studied so far there seems to be a force-independent component of active heat production. This component, which has been called the activation heat, was classically described by Hill (1949) and its magnitude has subsequently been measured in several ways (Hill, 1958; Gibbs, Ricchiuti & Mommaerts, 1966; Smith, 1972; Homsher, Mommaerts, Ricchiuti & Wallner, 1972; Rall, 1979, 1980). It is commonly believed that the magnitude of the activation heat indicates the amount of Ca^{2+} pumping undertaken by the sarcoplasmic reticulum to terminate a contraction (Chapman & Gibbs, 1972). From a knowledge of the sarcoreticular Ca^{2+} pump stoichiometry (Hasselbach & Oetliker, 1983) and the *in vivo* enthalpy of ATP hydrolysis it is therefore possible to estimate, from the activation heat, the Ca^{2+} flux per contraction (Chapman & Gibbs, 1972; Smith, 1972; Rall, 1982).

There is a tension-independent heat component in mammalian cardiac muscle (Gibbs, Mommaerts & Ricchiuti, 1967) and, as might be expected from a knowledge of the Ca^{2+} release cycle in cardiac muscle (Chapman, 1983), its magnitude is affected by changes in the extracellular Ca^{2+} level (Gibbs & Vaughan, 1968) and by several agents that alter contractility (see Gibbs, 1982 for a review). This supports the view that this component of energy expenditure reflects Ca^{2+} translocation processes in cardiac muscle as well, although there is some evidence that the Na^+–K^+ pump may also contribute to the tension-independent heat in cardiac muscle (Chapman, Gibbs & Gibson, 1970).

In skeletal muscle the most unambiguous estimates of the time course and magnitude of the activation heat have been made when preparations have been stretched to the point of zero overlap of the thick and thin filaments (Smith, 1972; Homsher *et al.* 1972; Rall, 1979). In cardiac muscle this technique is not successful since it is practically impossible to stretch cardiac sarcomeres beyond about 2·7 μm (Winegrad, 1974; ter Keurs, Rijnsburger, van Heuningen & Nagelsmit, 1980) without causing irreversible damage. In our experience attempts to eliminate active force production by stretching invariably damage the preparation (papillary muscles or trabeculae) so that upon return to l_{max}force production is noticeably impaired.

For this reason two main alternative methods of measuring the activation or stress-independent heat in cardiac muscle have evolved. In one, described by Gibbs *et al.* (1967), the preparation is shortened in steps to reach gradually a length at which no active force development is measured but where heat is evolved when the preparation is stimulated. In the other (see Alpert & Mulieri, 1982; Mulieri & Alpert, 1982), the hyperosmotic saline method of Hill (1958) has been used to abolish active force production. These methods provide estimates of the activation heat component which seem to be in reasonable agreement with one another, at least at temperatures close to 20 °C. Both, however, are open to criticism (see Discussion) and may not provide an accurate estimate of the true magnitude of the cardiac activation heat. For this reason we have recently used a quick-release technique to obtain another estimate of the activation heat. This method has been used before (Cooper, 1979) but it has not been employed to establish heat–stress (force/cross-sectional area) relations nor has it been employed to examine the effects of varying the extracellular

Ca^{2+} concentration. We also demonstrate in the present paper that many of our myothermic results are consistent with the data of Allen & Kurihara (1982) on the effects of muscle length on the Ca^{2+} (aequorin) transient in cardiac muscle.

Some aspects of these results have been reported in a preliminary form (Gibbs, 1985, 1986).

METHODS

Rabbits were killed by a blow to the neck and their hearts were rapidly removed and placed in cool Krebs–Henseleit solution. The coronary circulation was flushed with about 10 ml of cold (<4 °C) physiological solution and the hearts were placed in a dissection dish. Under aerated solution the wall of the right ventricle was reflected back and the papillary muscles were exposed. The right ventricular papillary muscle that best satisfied the demands of the myothermic technique (Gibbs, 1978) was selected and tied at either end (tendon or septal) with braided, non-capillary, 5/0 silk thread. The muscle was then placed under a resting tension of 5–10 mN before being transferred to the thermopile system where it was mounted vertically under a similar resting tension. The tendinous end of the preparation was connected to a thin tungsten wire, 70 mm long, that was attached to an ergometer lever.

The muscle thermopile system was enclosed in a glass chamber containing 35 ml of Krebs–Henseleit solution of the following composition (mmol l^{-1}): NaCl, 118; KCl, 4·75; $NaHCO_3$, 24·8; KH_2PO_4, 1·18; $MgSO_4$, 1·18; $CaCl_2$, 2·54; glucose, 11·0. The solution contained insulin (10 i.u. l^{-1}) and was gassed with 95 % O_2 and 5 % CO_2: its pH was 7·4. In some experiments the Ca^{2+} level was 0·25, 0·5 and 2 × that given above. All experiments were run at 27 °C.

In the present series of experiments twenty-three papillary muscles were used; their average mass (blotted wet weight), length (l_{max}) and cross-sectional area were 4·54 ± 0·36 mg; 6·07 ± 0·29 mm, 0·77 ± 0·06 mm^2 (mean ± S.E.M.) respectively. The average peak stress was 43·1 ± 3·1 mN mm^{-2} (measured at optimal length, l_{max}, in 2·5 mmol l^{-1} Ca^{2+} and in a train of thirty contractions at 1·0 Hz). The stress value is highly dependent upon cross-sectional area (see Fig. 1).

Heat measurements. These details have been fully described in earlier papers (Gibbs & Gibson, 1970; Loiselle & Gibbs, 1979) but a recent paper by Loiselle (1985) gives a very full description of the experimental set-up, calibration and heat-loss correction. In the current investigation several thermopiles have been used; two conventional electroplated thermopiles (Ricchiuti & Mommaerts, 1965), one vacuum deposition thermopile (Mulieri, Luhr, Trefry & Alpert, 1977) and one integrating thermopile (Wilkie, 1968). All heat records were corrected for the stimulus heat when that was of significant magnitude. In the majority of experiments this heat was negligible.

Because the latency release technique (see below) uses a release–stretch sequence which might artifactually generate heat either via a frictional effect or by altering basal metabolism, several release–stretch runs were always carried out in which the preparation was not stimulated but was cycled thirty times from l_{max} to l_{min} (the length at which no active stress was developed). There was no evidence of artifactual heat production.

Stimulation. The muscles were stimulated using a Digitimer D4030 plus an isolation unit. One electrode was always in the plane of the thermopile and one electrode cantilevered from the thermopile frame (Gibbs & Gibson, 1970). The stimulus rate during a train of thirty contractions was 1·0 Hz. The stimulus voltage was usually set at 7·0 V and the stimulus duration, with the solution drained, was in the 0·1–0·5 ms range.

Mechanical. All the experiments were carried out with the tendinous end of a papillary muscle attached, via tungsten wire, to the lever on a Cambridge Technology Model 300H ergometer. The use of this lever system necessitated some re-design of the muscle–thermopile chamber and the thermopile frames to minimize the distance between the lever and the preparation. The total compliance of ergometer, tungsten wire and silk ties was $1·6 \times 10^{-6}$ m mN^{-1}.

Experimental protocol. All measurements are the mean values per contraction of heat, force or length recorded in a train of thirty contractions at 1·0 Hz. After amplification all data were displayed on a Gould Mk 2400 pen recorder and were continuously sampled by a microprocessor data acquisition system and stored on floppy disks for subsequent computer analysis. After an equilibration period of 1–2 h, during which the muscles were continuously stimulated at a

frequency of 0·25 Hz, the length, l_{max}, at which maximum force development occurred was established and the preparations were then subjected to the following two procedures.

Progressive shortening protocol. In this protocol each muscle was progressively shortened down its length–isometric force relation, generally in six to ten steps. After equilibration at each new length isometric force and active heat production were measured in a train of thirty contractions ('down' run, see Gibbs & Gibson, 1970). In ten experiments the muscle was re-equilibrated for 20 min under solution at l_{max} and then immediately taken to l_{min} from where the converse experiment was carried out, i.e. the muscle was progressively extended in six to ten steps to l_{max} with mechanical and heat

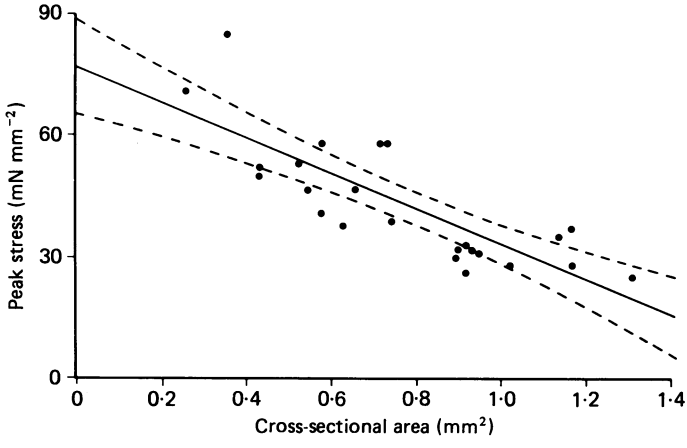

Fig. 1. Maximum stress (S_{max}, mN mm^{-2}) *versus* cross-sectional area (A, mm^2) for twenty-four papillary muscles. S_{max} determined in 2·5 mmol l^{-1} Ca^{2+} in a series of thirty contractions at a stimulation frequency of 1 Hz. $S_{max} = 76{\cdot}56 \pm 5{\cdot}49 - 43{\cdot}71 \pm 6{\cdot}74\ A$; $s_{y,x}$ (the standard error of regression) = 3·02; $r = -0{\cdot}80$. The dashed lines are the 95% confidence limits.

measurements being made at each length ('up' run). Since, for any given muscle, the heat–stress relations obtained in the 'down' and 'up' runs were not significantly different (see Results) in the remaining experiments a heat–stress relation was obtained in only one recording run. In half these cases a 'down' run was employed whilst in the remainder an 'up' run was used. Following the establishment of a heat–stress relation with the progressive shortening protocol the muscles were re-equilibrated under solution at l_{max} and then used in the latency release experiments (see below).

The heat–peak stress relation can be linear or curvilinear (Gibbs & Gibson, 1969; Mulieri & Alpert, 1982). A curvilinear heat–peak stress relationship can often be converted into a linear one if the active heat production is plotted against the total (active + passive) stress provided the passive stress does not exceed 15% of the peak stress at l_{max}. In both instances the intercepts of the heat–stress relation (tension independent or activation heat) are practically identical and in analysing the present experiments we have chosen, therefore, to use total stress and to fit the heat–stress data by linear regression. The conclusions reached in this paper about the activation heat are in no way altered by this method of analysis. It should be apparent that it is only the last few data points, in the high force range, where the stress values are altered somewhat (i.e. where passive stress is 5–15% of active stress development).

Latency release protocol. In this protocol the preparations were again stimulated to undergo trains of thirty contractions but were now released 15 ms after each stimulus (during the latency period) and held at different lengths for 500–550 ms before being returned (stretched) to their original length (l_{max}) prior to the next stimulus. The release rate (30 mm s^{-1}) was well above the maximum shortening velocity of these muscles. The release and stretch rates were identical. Similar results were obtained if the stimulus rate was decreased to 0·5 Hz. In the latter case the time spent at l_{max} was ~1500 ms rather than 500 ms: this means that the results are not critically dependent upon the length of time spent at l_{max} between beats (see also Cooper, 1979). In these latency release experiments the size of the release step was maximal for the first train of thirty stimuli (the length

step took the muscles to l_{min}) and was then progressively decreased such that the final measurement in the series was an isometric measurement, i.e. zero release contraction at l_{max}.

In twelve muscles the latency release experiments were repeated after the preparations had been equilibrated at l_{max} in Krebs–Henseleit solution containing 0·25, 0·5 or twice the normal level of Ca^{2+}. The equilibration periods were always 30–40 min long and in nine muscles the full series of experiments was completed. The Ca^{2+} sequence was always in the order 2·5, 0·625, 1·25 and 5·0 mmol l^{-1}. High Ca^{2+} levels prolonged contraction time and shortened the latency period. In the 5·0 mmol l^{-1} Ca^{2+} solution it was, therefore, occasionally necessary to slightly prolong the time at the short length (from 550–600 ms) and in two muscles it was necessary to start the release earlier (at 10 ms) to avoid the release taking place during the rise of active force production.

It took approximately 4 h to run all the release experiments at the various Ca^{2+} concentrations. At the completion of the experiments described above four preparations were returned to 2·5 mmol l^{-1} Ca^{2+} (normal) solution and equilibrated; they were then shortened to l_{min} for 20 min before a set of latency stretch experiments was run; i.e. stretches of progressively increasing extent were applied until l_{max} was reached.

In six preparations where only the progressive shortening down and latency release experiments in 2·5 mmol l^{-1} Ca^{2+} had been carried out, the muscles were equilibrated for 40 min in Krebs–Henseleit solution made hyperosmotic (2·5 × normal) by the addition of mannitol (see Alpert & Mulieri, 1982). During the hyperosmotic exposure period the muscles were stimulated at a low rate (0·2 Hz). At the end of the equilibration period the solution was drained and the active heat production was measured in response to ten stimuli at 0·2 Hz or thirty stimuli at 1·0 Hz (see Results).

Statistics. In each set of experiments, the activation heat was estimated in various ways within the same papillary muscle preparation. Each muscle thereby acted as its own control. In this way a potential source of variability due to differences in cross-sectional area between muscles (which markedly affects peak stress, see Fig. 1) was obviated. Data were analysed by analysis of variance for a repeated measured randomized-blocks design in which an individual muscle acted as a block. Differences in activation heat among muscles, could thus be separated from differences due to the method of estimation. Differences among mean values were then examined for statistical significance (at the 95% level of confidence) using the methods of Rodger (1975). Results are reported as mean ± S.E.M.

Where appropriate (in the progressive shortening and latency release series of experiments) each estimate of activation heat was determined as the intercept of the linear regression of active heat production *versus* total (active + passive) stress development. These intercept values then became the data for the analyses of variance. Analysis was achieved using the SAS (Statistical Analysis System) package on an IBM-4341 digital computer.

RESULTS

Activation heat estimated by muscle pre-shortening

In twenty-three muscles the mean activation heat, measured by gradually shortening muscles down their length–stress relation, was 1·94 ± 0·30 mJ g^{-1} blotted wet weight. A typical result is shown in Fig. 2 (continuous line). Because in ten experiments both a descending and ascending length–stress series were made quite separately (see Methods), it was possible to test for a difference in the resulting independent estimates of the activation heat. The descending series (l_{max}–l_{min}) yielded a mean value of 1·59 ± 0·26 mJ g^{-1} whereas the ascending series (l_{min}–l_{max}) yielded a value of 2·06 ± 0·57 mJ g^{-1} ($n = 10$). This difference is not statistically significant ($P > 0·2$).

Activation heat estimated by latency quick release

By varying the size of the length step (quick release), and measuring the resulting stress development and heat production at each new length, it is possible to

determine a heat–stress relation resulting from quick-release experiments in a manner analogous to that for pre-shortening the muscle. The only difference being that, in the quick-release protocol, activation of the preparation always occurs at l_{max}. A quick-release heat–stress relation is shown in Fig. 2 (dashed line). Using total stress (rather than active stress, see Methods) the heat–stress data for each muscle were analysed by linear regression. Once again, the intercept of this linear heat–stress relation estimates the activation heat. The mean value of activation heat so estimated was $3{\cdot}27 \pm 0{\cdot}40$ mJ g^{-1} ($n = 23$).

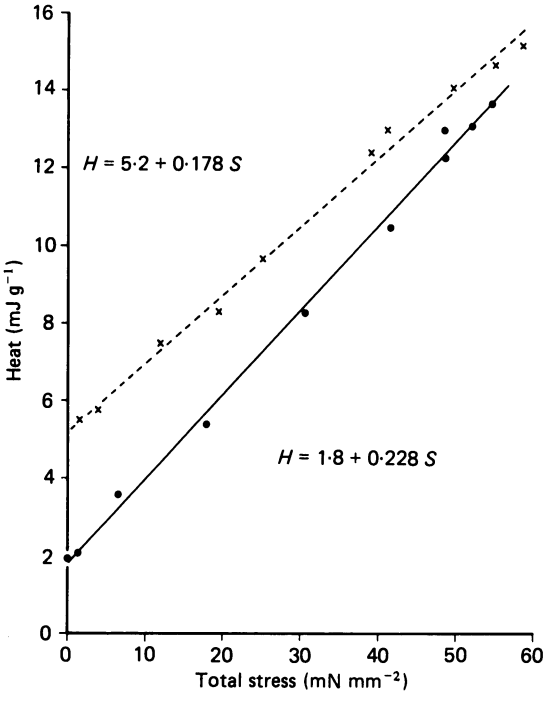

Fig. 2. A heat–total stress relation obtained by shortening a papillary muscle in gradual steps (continuous line) compared with a heat–stress relation obtained by the quick-release technique (dashed line). The muscle weighed 2·9 mg and was 4·7 mm long at l_{max}. H, heat; S, stress.

This linear regression estimate was very close to the directly measured activation heat value obtained by rapidly shortening the muscle in a single step from l_{max} to l_{min} during the latency period ($3{\cdot}46 \pm 0{\cdot}39$ mJ g^{-1}) but is 79% greater than the value estimated ($1{\cdot}94 \pm 0{\cdot}30$ mJ g^{-1}) by gradually pre-shortening the muscle to l_{min}. The difference between the two latency release estimates is not statistically significant but both values are significantly greater than the estimate arising from the intercept of the heat–stress relation obtained by gradual pre-shortening (for details of analysis see Methods: Statistics). Results for a single experiment in which the activation heat was estimated by the latency release and pre-shortening method are shown in Fig. 3. It is important to note that the heat records shown in Fig. 3 represent the summed total heat (initial + recovery), corrected for heat loss, in the train of thirty contractions. This total heat is given by the amplitude of the trace above the baseline

once the trace has levelled out. In the text and Table the heat data are presented as the total heat per contraction. The mechanical records shown in Fig. 3 are from a single contraction (stimulus) cycle within the train.

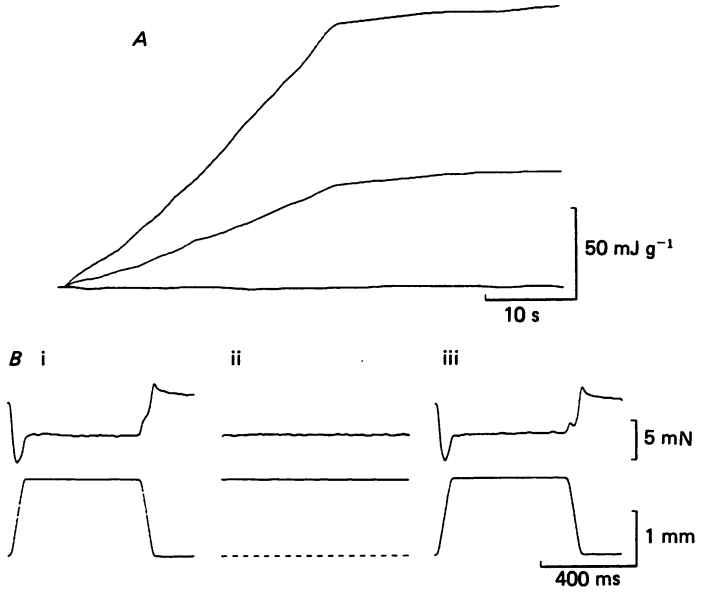

Fig. 3. Experimental traces showing total heat production in thirty contractions at 1·0 Hz (*A*) and single contraction mechanical records (force and length) in progressively shortened and latency release experiments (*B*). The top trace of *A* shows the summed total heat production in a train of thirty contractions where the muscle was released from l_{max} to l_{min} 15 ms after each stimulus; the middle trace shows the heat produced by the same muscle in a train of thirty contractions after it had been pre-shortened to l_{min}; and the bottom trace records the heat production in thirty release–stretch cycles but where there was no muscle stimulation. *B* shows the associated mechanical responses (force above, length below – muscle shortening being represented by an upward deflection of the trace) from a single contraction (stimulus) cycle within the train. *B*i shows the normal latency release mechanical transients, *B*ii shows the lack of a force response in a muscle pre-shortened to l_{min} and *B*iii shows the force and length steps in an unstimulated preparation subjected to the release–stretch cycles as in i. The papillary muscle weighed 4·3 mg and was 4·7 mm long at l_{max}.

Activation heat as a function of extracellular Ca^{2+}

In nine papillary muscles the latency release technique was used to determine the heat–stress relation at four different levels of extracellular Ca^{2+}. The Ca^{2+} exposure sequence was always in the order: 2·5, 0·625, 1·25 and 5·0 mmol l^{-1}. A typical experimental result is shown in Fig. 4.

The repeated-measures nature of the experimental design (see Methods: Statistics) greatly increased the statistical power to detect differences among means. Hence, despite the considerable degree of variation among muscles (see Table 1) each mean estimate of the activation heat (intercept of linear heat–stress relation: *a* in Table 1) differed from each other one across the four Ca^{2+} treatments. That is, the external

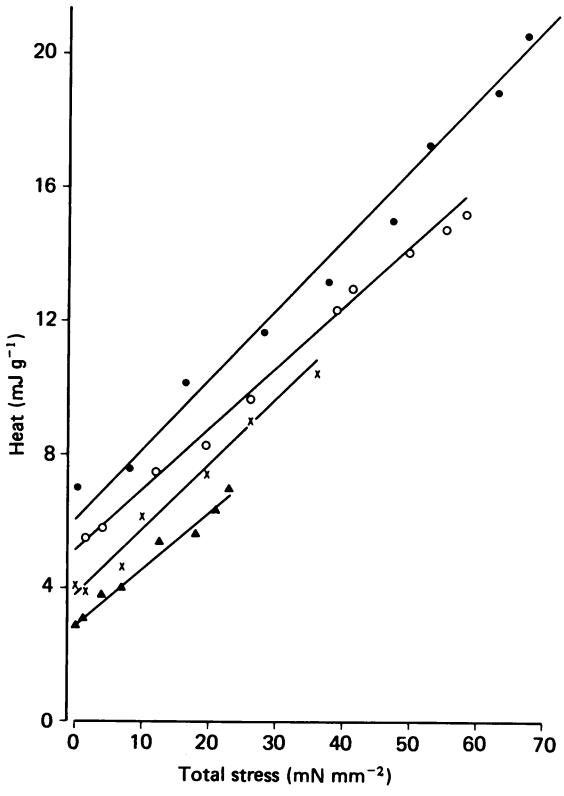

Fig. 4. Heat–total stress relations obtained using the quick-release technique in four different Ca^{2+} concentrations (●, 5; ○, 2·5; ×, 1·25; and ▲, 0·625 mmol l^{-1}). The muscle is the same one as in Fig. 2.

TABLE 1. Effect of extracellular Ca^{2+} on peak total stress and the heat–stress relation

	S_{max}	a	b
Calcium concentration (mmol l^{-1})			
5·0	57·6±7·0	4·15±0·51	0·160±0·011
2·5	44·1±4·6	3·34±0·41	0·156±0·015
1·25	33·7±4·0	2·98±0·29	0·146±0·014
0·625	22·9±3·7	2·58±0·24	0·141±0·016

S_{max} is the peak total stress in mN mm^{-2}, a is the intercept (activation heat) in mJ g^{-1} and b the slope of the heat–stress relation.

Data presented as mean±S.E.M., $n = 9$.

Ca^{2+} concentration significantly affected the measured activation heat; the mean values were: 4·15, 3·34, 2·98 and 2·56 mJ g^{-1} for 5, 2·5, 1·25 and 0·625 mmol l^{-1} Ca^{2+} respectively.

Because the extracellular Ca^{2+} level affects both contractility and the magnitude of the activation heat (S_{max} and a; respectively, in Table 1), it is instructive to plot the activation heat *versus* peak stress relation. This has been done in Fig. 5. This Figure differs from the heat–stress relations shown above in that stress has been varied by varying the external Ca^{2+} concentration rather than by varying muscle

length in some manner. The data are well-fitted by linear regression (activation heat, $H_a = 1{\cdot}22 \pm 0{\cdot}275 + 0{\cdot}0514 \pm 0{\cdot}006\,24\ S_{max}$ mJ g^{-1}, $r = 0{\cdot}81$; the standard error of regression, $s_{y,x} = 0{\cdot}719$; $n = 36$). The intercept of this relation ($1{\cdot}22 \pm 0{\cdot}28$ mJ g^{-1}) is significantly greater than zero and the implications of this are considered in the Discussion.

The effect of prolonged pre-shortening

It is clear from the results of Allen & Kurihara (1982) that the Ca^{2+} transient is depressed in a muscle that has spent a lengthy period at a short length. In order to see whether we could detect this effect in rabbit cardiac tissue, papillary muscles were

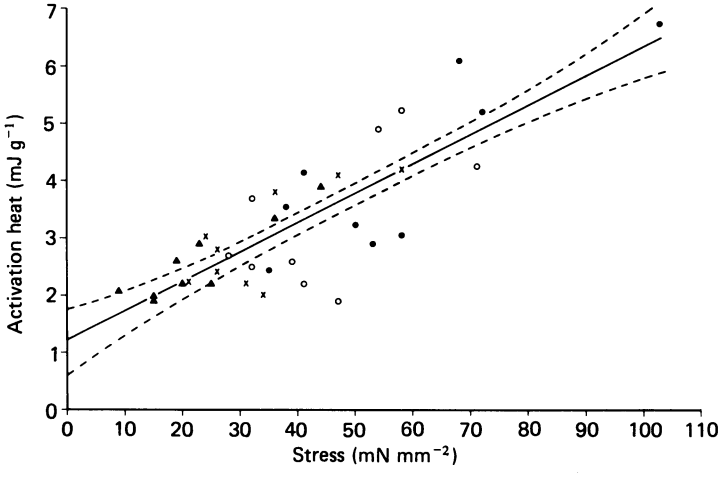

Fig. 5. Activation heat, determined by the latency quick-release technique, plotted against peak total stress. Data obtained from nine muscles each equilibrated in four different Ca^{2+} solutions (●, 5; ○, 2·5; ×, 1·25; and ▲, 0·625 mmol l^{-1}).

left to equilibrate (under solution containing 2·5 mmol l^{-1} Ca^{2+}) at l_{min} for 20 min. The converse of the above latency release experiments were then performed. That is, the muscles were stimulated and 15 ms later stretched and held at a new length for 500 ms before being returned to l_{min}. It was hoped that by varying the magnitude of the stretch step, a complete heat–stress relation could be established. Unfortunately it generally proved impractical to fully stretch the muscles to l_{max} because, after 20 min equilibration at l_{min}, the large stretches caused larger increases in passive tension than expected. These increases in passive tension concomitantly increased the resting heat rate, thereby altering the thermal baseline upon which the measurement of active heat production is made. The effect of passive tension upon the resting heat rate of cardiac muscle has been described previously (Gibbs *et al.* 1967; Loiselle, 1985).

The resting heat rate following stretches to l_{max} did, however, remain sufficiently stable in four preparations for this protocol to be completed. In these four preparations, the mean activation heat measured after pre-equilibration at l_{min} was $1{\cdot}78 \pm 0{\cdot}04$ mJ g^{-1} whereas the corresponding value determined for the same four muscles using the quick-release technique was $3{\cdot}38 \pm 0{\cdot}70$ mJ g^{-1}. Thus a 20 min pre-

equilibration period at l_{min} caused a statistically significant depression of the measured activation heat.

Activation heat estimated by the hyperosmotic method

Originally we had intended to record the heat liberated in a train of thirty twitches at 1·0 Hz with the muscle equilibrated in hyperosmotic mannitol. However, this planned stimulus regime always produced a mechanical response that resembled that observed in an imperfectly fused tetanus. The force level was usually only about 10% of that seen in a normal twitch but on one occasion was almost 30% of the twitch response. Whenever this maintained force response was observed the active heat production was high. In five of the six preparations studied, this summation phenomenon could be avoided by lowering the stimulus rate to either 0·5 or 0·33 Hz. When the stimulus rate was reduced to 0·2 Hz, the rate used by Alpert & Mulieri (1982), then there was never any evidence of summation and twitch force levels were very low (1–2 mN mm^{-2}).

Hence it was decided to estimate the activation heat in hyperosmotic mannitol by averaging over ten contractions at a stimulus frequency of 0·2 Hz. The mean value under these conditions was $2·03 \pm 0·12$ mJ g^{-1} which was not significantly different from the value estimated by the pre-shortening method ($1·78 \pm 0·28$ mJ g^{-1}) in the same six preparations. In the same preparations, the latency release method yielded a value of $2·93 \pm 0·39$ mJ g^{-1}, which is significantly higher than that obtained with either of the other methods.

DISCUSSION

The data presented in this paper make it clear that there is no unique method for measuring activation heat in cardiac muscle. Even if it were possible to stretch cardiac muscle such that it was on the descending limb of the sarcomere length–tension relation it is not clear what the relevance of the measured activation heat would be in relation to a contraction at l_{max}.

Comparison of different methods of measuring activation heat

The results obtained in this study strongly suggest that progressively shortening a muscle to a length where it cannot develop tension leads to an estimate of activation heat that is too low in relation to a contraction at l_{max}. There have always been two major objections to the use of this method. Firstly it can be argued that the heat will be overestimated because there will still be some cross-bridge activity at the short length (l_{min}) even though this is not translated into measurable force at the transducer. Such activity might even take place against some internal load (Gordon, Huxley & Julian, 1966). Secondly, since the mid 1970's the evidence has been growing that when cardiac muscle is shortened Ca^{2+} release is compromised (Fabiato & Fabiato, 1975; Jewell, 1977). It seems clear from our data that the second effect is the more serious one in terms of estimating the activation heat in a contraction at l_{max} by this method.

Having said that, it should be pointed out that in comparative studies (e.g. varying Ca^{2+}, temperature, or using inotropic agents) the new heat–peak stress

relation is virtually moved parallel to the control relation at all stress levels (see Gibbs, 1978) and the heat displacement at l_{max} is quite similar to the heat displacement at the zero-tension intercept (l_{min}).

It may of course be argued that the other method we have used here, the latency quick release method, may have its own drawbacks and we need to consider them. Firstly does the rapid release still inactivate Ca^{2+} release in the manner that the progressive shortening protocol does. The most direct evidence, that of Allen & Kurihara (1982), would say not (see their Fig. 2). They show in fact that the Ca^{2+} transient is little affected by even 2 min at a new shorter length. It needs about 8 min or more before the magnitude of the Ca^{2+} transient is decreased; Allen and Kurihara's 'steady-state' procedure.

If inactivation of Ca^{2+} release does not occur during the quick release can we also dismiss the possibility that the quick-release technique is overestimating the magnitude of the activation heat component? Here the evidence can only be indirect and we need to consider three major possibilities.

(i) That at the short length (l_{min}) there is still ATP usage attributable to residual cross-bridge activity. This possibility cannot be ruled out as there is some macroscopic muscle shortening even in contractions where the detected twitch force is essentially zero. We believe, however, that this effect is energetically insignificant in that there is usually little difference between the directly measured activation heat (i.e. in a release from l_{max} directly to l_{min}) and the value obtained by extrapolation of the latency release heat–stress relation to zero stress, even if only data is used in which the length step is less than half the l_{max}–l_{min} distance.

(ii) That shortening heat (Hill, 1949) contributes to the heat measured at short lengths following a quick release. All the available evidence suggests that if there is a shortening heat component to the cardiac energy balance sheet it must be very small. It has proved to be impossible to experimentally superimpose heat traces recorded in isotonic and isometric contractions and to show that the rate of heat production in an isotonic contraction ever exceeds that measured during the rising phase of an isometric contraction at l_{max} (see for example Fig. 5, Gibbs & Gibson, 1970). In whole-heart oxygen consumption experiments Weber & Janicki (1977) concluded that muscle fibre shortening presented a negligible cardiac energy cost and it is our belief that the pressure–volume area isoefficiency data of Suga and colleagues (Suga, Hayashi & Shirahata, 1981) is incompatible with a shortening heat of significant magnitude. It is well established that force (stress) is the major mechanical determinant of cardiac energy flux (for review, see Gibbs, 1978), and this conclusion is not seriously questioned even for skeletal muscle (Rall, 1978). We believe, therefore, that in the latency release experiments where the release rate exceeds the preparation's maximum shortening velocity, and where the externally measured force was zero, any shortening heat component would be insignificant.

(iii) That the rapid length change itself triggers additional Ca^{2+} release above that normally released upon stimulation at l_{max}. Allen & Kurihara (1982) did report some quick-release experiments which suggested that there was a short-lived increase in the $[Ca^{2+}]_i$ following a release. Their releases were however made 110 ms into the contraction when stress development was already at least one-third of maximum. Allen and Kurihara's conclusion was that the binding constant of troponin for Ca^{2+}

is increased as tension develops (Bremel & Weber, 1972) and hence the most likely reason for the increased Ca^{2+} transient following a quick release was release of previously bound Ca^{2+} from troponin, i.e. these authors were not suggesting that additional Ca^{2+}, above that normally mobilized for contraction, was being released and this interpretation has been confirmed recently (Kentish & Allen, 1986).

For all the reasons outlined above we believe that the latency release method is a suitable way of measuring activation heat in cardiac muscle. Our quick-release and shortening data can be compared with those of Cooper (1979) who used a somewhat similar protocol in measurements of the oxygen consumption of cat papillary muscles at 29 °C. In this polarographic study Cooper employed releases starting at time zero using 120 contractions at a stimulus frequency of 0·5 Hz. His latency release value for activation heat was about 2·0 mJ g^{-1} whilst his progressive shortening down estimate was 1·1 mJ g^{-1} (assuming the calorific equivalent of oxygen usage to be 20 kJ l^{-1}). Clearly Cooper has shown the deactivation effect of progressive shortening down but his values, with both protocols, are low relative to ours; indeed he suggested that the activation component would only account for about 14% of the total twitch energy. The reason for the discrepancy is not clear and we must bear in mind that the comparison is across species and that the release time and release rate are somewhat different. None the less these differences are not adequate, in our minds, to account for the quantitative discrepancy between the values reported here and those of Cooper.

The difference between the shortening down results and the latency release results are probably due to the effects of preparation length on the magnitude of the activation heat. These effects are large and suggest that there is less Ca^{2+} release and hence uptake at short lengths. In this respect our results are in accord with the data of Allen, Jewell & Murray (1974), Fabiato & Fabiato (1975), Jewell (1977) and Allen & Kurihara (1982). We have shown that a 'down' run, i.e. l_{max} to l_{min} leads to an underestimate of the activation heat and that a long period of time (20 min) spent at a length shorter than l_{max} can halve the activation heat magnitude (latency stretch experiments).

Activation heat can be measured under conditions that uncouple the Ca^{2+} release cycle from the cross-bridge cycle and there are a variety of methods that have been used (stretch, shortening, tonicity, D_2O). The effect of hyperosmotic solutions was first investigated by Hill (1958) but, although twitch tension was abolished, it was clear from subsequent work (Howarth, 1958) that there was still cross-bridge activity occurring. In addition there has, as yet, not been any clear demonstration in cardiac muscle that hyperosmotic treatment leaves the normal Ca^{2+} release cycle unchanged. It seems clear to us that in physiological solutions, made hyperosmotic by mannitol, there is definitely cross-bridge activity that is easily demonstrated at higher stimulus frequencies. The data we have obtained at low stimulus rates (0·2 Hz) is in good agreement with data reported by Alpert & Mulieri (1982). These authors, in agreement with other aspects of our results, report that shortening down a muscle leads to lower activation heat values than that recorded at l_{max} in mannitol (see Fig. 17, Mulieri & Alpert, 1982).

We have shown previously that the magnitude of the activation heat decreases as the extracellular Ca^{2+} concentration is lowered (Gibbs & Vaughan, 1968). In that

study it was reported that lowering the extracellular Ca^{2+} by 75% reduced the activation heat by 29%. Placement of the papillary muscles into Ca^{2+}-free solution (chelating agents present) still left the activation heat magnitude at about 40% of the control (2·5 mmol l^{-1} Ca^{2+}) value even when peak tension at l_{max} was less than 10% of normal.

The latency release data presented in this paper show once again that the magnitude of the activation heat is dependent upon the extracellular Ca^{2+} but the relationship is not 1:1 suggesting that the origins of this component are complex. An examination of our pooled data across all muscles and all treatments (Ca^{2+} levels) shows that there is a relationship between peak stress and activation heat (see Fig. 5) and also shows that at zero stress there is still a sizeable residual heat component. This heat component could perhaps be ascribed to Na^+–K^+ pump activity since we have shown that the magnitude of the tension-independent heat is increased not only by raising extracellular Ca^{2+} levels but also by raising extracellular Na^+ (Chapman *et al.* 1970). Based on some estimates of the Na^+ flux during a cardiac action potential we have calculated that the Na^+–K^+ pump could expend in excess of 1·0 mJ g^{-1} of energy per contraction (Gibbs & Chapman, 1979). None the less additional experiments are needed to firmly establish the potential contribution of Na^+–K^+ pump activity to the tension-independent heat in cardiac muscle.

Activation heat and the amount of Ca^{2+} release

The magnitude of the activation heat component we have measured may seem high relative to the amount of Ca^{2+} released into the sarcoplasm. We have previously calculated (see Gibbs & Chapman, 1979) that if at full activation 90–100 nmol of Ca^{2+} ions are needed per gram of tissue (Solaro, Wise, Shiner & Briggs, 1974) and if the pump works with a stoichiometry of two Ca^{2+} per ATP (Hasselbach & Oetliker, 1983) then at maximum activation about 4 mJ g^{-1} of heat would be measured: this value includes recovery heat arising from the replenishment of high-energy phosphates by oxidative metabolism. Now the 90–100 nmol g^{-1} figure (Solaro *et al.* 1974) is based on the binding capacity of isolated myofibrils, the myofibrillar ATPase activity and the force generation of chemically skinned papillary muscles as a function of free Ca^{2+}. A recent study by Pierce, Philipson & Langer (1985) using rabbit ventricular tissue suggests that the above value is far too low because no allowance was made for the Ca^{2+}-buffering capacity of other cellular components; a similar conclusion can be drawn from data in the review of Fabiato (1983). When the passive Ca^{2+}-binding capacity of a soluble and particulate fraction of a ventricular homogenate was assessed the conclusion was that the Ca^{2+}-buffering capacity of the myocardial cell is three- to fivefold higher than previously estimated and the authors conclude that to achieve even half-maximal myofibrillar ATPase activity more than 100 nmol total Ca^{2+} g^{-1} wet weight would be needed to achieve free Ca^{2+} concentrations in the 2–10 μmol l^{-1} range. Obviously this data, obtained in the same animal species that we are using, would be consistent with our myothermic results and suggests that under our experimental conditions there is just under half-maximal myofibrillar ATPase activity. This would support the suggestion that mammalian cardiac muscle normally functions at submaximal levels of activation (Fabiato, 1981).

We conclude that there is encouraging agreement between our myothermic data on cardiac activation heat and recent experiments that describe the length dependence of Ca^{2+} release in cardiac muscle. The data also seem to be in reasonable quantitative agreement with recent estimates of Ca^{2+} release under normal physiological conditions. The latency quick-release method described in this paper raises the estimate of the activation heat to values that can account for 25–30% of the total active energy flux per beat; a fraction comparable to that measured in skeletal muscle. The results also, however, stress that the cardiac activation heat magnitude is continuously variable by factors such as extracellular Ca^{2+} levels and length, factors that have comparatively little effect on the magnitude of skeletal muscle activation heat.

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