Pflügers Arch (1985) 405:155–162

Pflügers Archiv
European Journal of Physiology

The rate of resting heat production of rat papillary muscle

Denis S. Loiselle

Department of Physiology, Monash University, Clayton, Victoria 3168, Australia

Abstract. The rate of resting heat production of quiescent rat left ventricular papillary muscles was measured myothermically. The effects of contractile activity, stretch, oxygen partial pressure, temperature, amino acids and time were examined. The rate of basal heat production was the same throughout the day whether or not muscles contracted isotonically under a small pre-load. Passive stretch increased the rate of resting heat production; the stretch-induced increment was highly variable from muscle to muscle. The resting heat rate per se was only moderately sensitive to oxygen partial pressure and temperature, and was insensitive to the presence of amino acids in the bathing medium. The stretch-induced increase in resting heat rate was independent of these three factors. The rate of resting heat production declined exponentially with time to reach a plateau about 4 h following cardiectomy.

Key words: Cardiac basal metabolism – Temperature effect – Stretch effect – Amino acids – Oxygen partial pressure

Introduction

Under normal physiological conditions cardiac muscle never undergoes lengthy periods of rest. It has, nevertheless, a resting or basal metabolic rate which can be examined by rendering the muscle inactive. The metabolic rate of quiescent cardiac muscle is, like the mitochondrial content (Page et al. 1971; Delbridge and Loiselle 1981), about five times higher than that of skeletal muscle (Gibbs 1978). Indeed, in the rat it may account for over one-third of the total cardiac metabolism when the animal is at rest (Loiselle and Gibbs 1979).

The cause of the high basal metabolic rate of cardiac muscle remains unknown. In vitro the rate varies inversely with muscle diameter (Loiselle and Gibbs 1983) in much the same manner as the active stress (force per cross-sectional area) production (Delbridge and Loiselle 1981). This dependence seems unrelated to the general question of oxygen sufficiency of isolated papillary muscles (Loiselle 1982) because, in contrast to predictions of the anoxic core hypothesis (Hill 1928; Whalen 1960; Cranefield and Greenspan 1960; Whalen and Fangman 1963), the stretch-induced increment in resting metabolism is unrelated to either muscle diameter or, equivalently, the metabolic rate per se (Loiselle and Gibbs 1983). In species with low rates of cardiac resting metabolism, the observed stretch-induced increment (Cranefield and Greenspan 1960; Lee 1960; Whalen 1960; Gibbs et al. 1967; Pool and Sonnenblick 1967; Loiselle 1982; Loiselle and Gibbs 1983) is typically in excess of that predicted by either the early Hill (1928) model or by a more realistic model (Loiselle 1982) which assumes some dependence of oxygen consumption upon concentration. It is thus of importance to re-examine the effect of stretch in a species with an inherently high rate of resting cardiac heat production. If the stretch effect is of purely geometric origin, reflecting increased oxygen consumption subsequent to reduction of a putative hypoxic core region, then it should be greater the higher the rate of basal metabolism prior to the stretch.

The effect of oxygen partial pressure upon basal metabolism likewise has not been examined in a species with an inherently high rate of cardiac basal metabolism. Whereas the oxygen tension seems to have little influence on resting cardiac metabolism in either dog Purkinje fibres (Greenspan and Cranefield 1963) or rabbit papillary muscles (Loiselle and Gibbs 1983), both of these species have a low rate of resting cardiac metabolism vis-a-vis the rat (Loiselle and Gibbs 1979). The high rate of resting cardiac heat production of the rat might be expected to decline in low partial pressure of oxygen. It might be further expected that such an effect would be enhanced by temperature, although there exists some evidence of a low Q_{10} of cardiac basal metabolism in vitro (Lochner et al. 1968; Loiselle and Gibbs 1983).

The effect of amino acids upon cardiac basal metabolism has not been extensively examined. The rate of uptake of amino acids is enhanced by stretch in both isolated rabbit papillary muscles (Lesch et al. 1970; Peterson and Lesch 1972) and in isolated perfused rat hearts (Morgan et al. 1983; but see Hjalmarson and Isaksson 1972). Both the rate of resting heat production (Loiselle and Gibbs 1979) and the rate of turnover of cardiac proteins (Earl et al. 1978) is high in the rat. It is thus of interest to test the effect of amino acids upon both the rate of basal heat production per se and upon the stretch effect in this species, particularly in view of the suggestion by Gibbs (1978) that the high cardiac basal metabolism may reflect the high rate of protein turnover.

Finally, this study was motivated by the possibility that a previous report (Loiselle and Gibbs 1983) might have underestimated the effect of temperature on cardiac basal metabolism. The reported Q_{10} values (1.28–1.42) were sufficiently low to warrant a re-appraisal of the possible errors involved in the myothermic determination of resting metabolic rate. The possibility of temperature-dependent errors arises from the fact that the resting heat production (unlike its active heat counterpart) must be made with refer-

Offprint requests to: D. Loiselle, Department of Physiology, School of Medicine, The University of Auckland, Private Bag, Auckland, New Zealand

Table 1. Muscle characteristics

Muscle	W_B	W_H	w	l	HL	$\Delta l/l$	d_c	A_c
1	280	0.80	4.2	6.5	0.12	0.31	0.91	0.65
2	235	0.70	6.8	8.0	0.10	0.21	1.04	0.85
3	350	0.88	5.3	7.5	0.13	0.24	0.95	0.71
4	330	0.77	7.1	8.0	0.13	0.26	1.06	0.89
5	265	0.85	6.0	8.5	0.15	0.14	0.95	0.71
6	225	0.63	4.9	5.5	0.13	0.32	1.07	0.89
7	195	0.61	3.0	5.5	0.12	0.28	0.83	0.55
8	215	0.57	2.0	6.0	0.17	0.20	0.65	0.33
Mean	262	0.73	4.9	6.9	0.13	0.24	0.93	0.70
SEM	20	0.04	0.6	0.4	0.01	0.02	0.05	0.07

W_B, rat body weight (g); W_H, heart weight (g); w, muscle weight (mg); l, muscle length (mm) under a 1 g pre-load; HL, rate of heat loss (s^{-1}) from the drained muscle/thermopile system (see Methods); $\Delta l/l$, maximum relative extent of lengthening under a 12 g pre-load. A_c; d_c,calculated muscle cross-sectional area (mm^2) and diameter respectively assuming the muscle is a cylinder of unit specific gravity; SEM = standard error of the mean

ence to an absolute zero output of the active thermocouple junctions. In the current study numerous precautions were taken in order to assure that this null output, together with baseline stability and evaporative heat loss from the thermopile, was independent of temperature.

Methods

Adult Sprague-Dawley rats of either sex were killed by a blow to the head. The heart was quickly removed and placed in pre-warmed (35° C) modified Krebs solution aerated with 95% O_2/5% CO_2. The aorta was back-perfused until the coronary exude appeared clear. The apex of the left ventricle was incised and the wall reflected exposing the two left ventricular papillary muscles. A muscle (details given in Table 1) was selected and tied at either end with braided, siliconized, non-capillary silk thread (Ethicon 5/0). By attaching the ties to either end of a small C-shaped spring, the muscle was removed under a tensile force of about 1 g and mounted vertically on the thermopile (see Fig. 1).

The muscle-thermopile system was enclosed in a glass chamber containing 55 ml of modified Krebs solution of the following concentration (mmol · l^{-1}): NaCl 118, KCl 4.75, $NaHCO_3$ 24.8, $MgSO_4$ 1.18, KH_2PO_4 1.18 and $CaCl_2$ 2.54. The metabolic substrate was pyruvate (10 mmol · l^{-1}) and insulin (20 U · l^{-1}) was provided. The pH was 7.3. Experiments were conducted at both 20° C and 27° C. The muscle chamber was aerated with either 95% O_2/5% CO_2 or 45% O_2/5% CO_2/50% N_2. Amino acids, when present, were added to the Krebs solution in the form of 20 ml Aminosol 10% (Vitrum, Stockholm) per l of Krebs solution. This yielded the following L-amino acid concentrations (mmol · l^{-1}): isoleucine 56, leucine 87, lycine 51, methionine 30, phenylalanine 42, threonine 37, valine 44, tryptophan 54, alanine 32, arginine 17, aspartic acid 45, cystine 9, cysteine 19, glutamic acid 154, glycine 29, histidine 17, proline 82, serine 71 and tyrosine 38.

Heat measurements. The rate of resting heat production was measured using two wire-wound thermopiles having slightly concave surfaces to accommodate a papillary muscle. The active regions of the thermopiles contained about 100 constantan-silver (electroplated) junctions in a length of 7 mm. Sensitivity of the thermopiles was approximately 2.5 mV/° C. Signals were amplified by an Astrodata Model

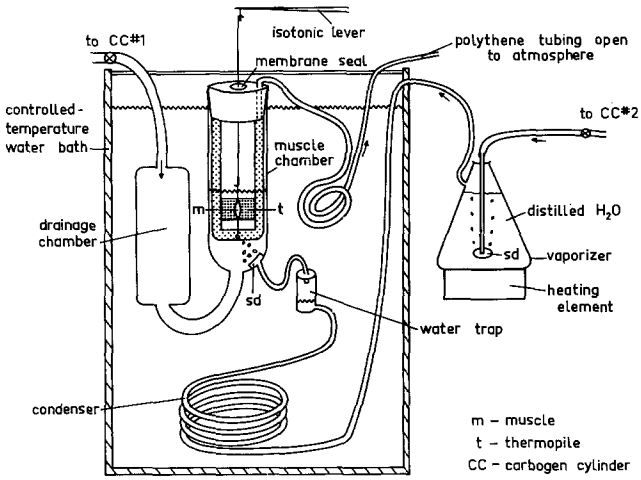

Fig. 1. Schematic diagram of the muscle-thermopile chamber with its associated aeration, humidification and temperature-stabilization apparatus. A steady supply of carbogen from *CC* (carbogen cylinder) 2 passes, via a fine sintered disk (*sd*), through the vapouriser (80° C), condensor (at bath temperature) and water-trap into the Krebs solution bathing the muscle (*m*) resting on the thermopile (*t*). In order to record muscle heat production, the flow of carbogen from CC 1 is interrupted and Krebs solution flows into the drainage chamber. The polythene tubing acts as a dead-space for humidified carbogen (rather than room air) to be drawn into the muscle-thermopile chamber if the rate of drainage exceeds the rate of carbogen flow from CC 2. Entire apparatus, except for vapouriser and isotonic lever, immersed in a temperature-controlled water bath

120 Nanovolt Amplifier, passed through an electronic network which corrected for the (exponential) rate of heat loss (Gibbs and Gibson 1969, 1972), and displayed on a Gould Brush 260 chart recorder. A number of modifications to the previous method of aerating the muscle-thermopile chamber were made in order to minimize the risk of underestimating the effect of temperature on basal heat production (see Introduction). These modifications will be described in some detail.

Temperature stabilization. The thermopile together with the muscle was enclosed in one arm of a glass U-tube which contained the Krebs solution. The column of Krebs solution

was raised to envelope the thermopile by a compressed mixture of carbogen (95% O_2/5% CO_2) from a cylinder. When making heat measurements this carbogen source was disconnected and the solution drained into the other arm of the U-tube. Thus one carbogen cylinder (CC 2, Fig. 1) supplied a continuous flow of carbogen to the Krebs solution at a constant rate of 25 ml/min. Since the U-tube containing the muscle-thermopile system had to be open to atmosphere in order to permit drainage of the Krebs solution prior to making heat measurements, the carbogen stream from the second cylinder assured that room air did not dilute the oxygen concentration within the muscle-thermopile chamber upon draining. In order to ensure that the flow of carbogen did not act as a heat sink it was passed through a fine-bore sintered disk into a reservoir of distilled water maintained at 80–85° C (the vapouriser, Fig. 1) and thence through 10 m of stainless-steel tubing and a water-trap (the condensor, Fig. 1) before passing through a second fine-bore sintered disk in the side of the muscle chamber (U-tube). The vapouriser raised the partial pressure of H_2O of the carbogen stream well above the saturation pressure at 27° C and the condensor assured that the temperature of the carbogen stream was reduced back to bath temperature before entering the muscle chamber. The continuous slow accumulation of liquid H_2O in the water-trap at experimental temperatures gave assurance that the carbogen stream entering the muscle chamber was fully water-saturated and hence caused no evaporative heat loss from the muscle-thermopile system.

The port at the top of the thermopile, through which passed a stainless-steel rod which connected the muscle to the isotonic lever (Fig. 1), was closed to the atmosphere by a tightly fitting (dental dam) membrane held in place by a heavy brass ferrule. To permit drainage of the muscle chamber and the making of heat measurements, a second port was opened into the muscle-thermopile chamber. This port was open to atmosphere through 2 m of polythene hose all but the final few centimetres of which were immersed in the water bath. This served as a "dead-space" of humidified, temperature-controlled carbogen which could be drawn back into the chamber upon draining (if the rate of draining exceeded the rate of carbogen flow from the second cylinder) and hence prevented the admixture of room air with carbogen in the muscle chamber.

The U-tube muscle chamber containing the muscle-thermopile system was lowered into a covered 15 l water bath. The water in this temperature-controlled bath was vigorously stirred by a stream of compressed air and circulated through a Haake Model N3 Ultrathermostat operating in series with a Haake Model K11 Refrigeration Unit.

Reliability and validity of the heat measurements. Unlike the measurement of any component of active heat production of muscle, the measurement of resting heat must be made with reference to an absolute base-line. This base-line is taken as the output of the thermopile alone when it is under solution. Ideally, draining the solution from the thermopile at any temperature, in the absence of a muscle, should lead to zero output. If the carbogen stream is either not equilibrated to bath temperature or is not fully water-saturated, or if room air enters the muscle chamber upon draining, then the output of the thermopile will not be zero and may be bath temperature-dependent. The modifications described above were made in an attempt to prevent these possible sources of error.

The reliability of temperature stability and the validity of the heat measurements were examined by comparing the thermopile output upon draining at 20° C and 27° C. This was done both with the thermopile alone and with an "artificial muscle" (a cylinder of papillary muscle proportions, made of 2% agar) mounted on the thermopile. Despite all the precautions outlined above, a small but consistent difference in thermopile output at 20° C and 27° C resulted upon draining. The average results of 10 experiments were 1.10 ± 0.11 μV at 20° C and 0.63 ± 0.23 μV at 27° C. This draining artifact was not influenced by the presence of an agar "artificial muscle" on the thermopile. Likewise the effect was independent of the temperature of the vapouriser (in the range 60° C to 90° C) and of the rate of flow of the carbogen stream (in the range 10 to 50 ml/min). Vapouriser temperature and carbogen flow rate were standardized at 80° C and 25 ml/min respectively and correction factors corresponding to 1.10 μV at 20° C and 0.63 μV at 27° C were applied to the measured resting heat values. This correction procedure had the effect of slightly increasing the measured difference in heat rates at the two temperatures and hence of slightly inflating the calculated Q_{10} value.

Rate of heat loss. As described previously (Gibbs and Gibson 1969, 1972), the rate of heat loss from the muscle-thermopile system was essentially exponential. Experiments with agar "artificial muscles" showed that the rate of heat loss was independent of bath temperature. These experiments were repeated with three papillary muscles with the same result; relative discrepancies ranged from 0 to 2.6%. In addition, the rates of heat loss from each of four papillary muscles were measured separately at each of two muscle lengths (corresponding to pre-loads of 1 and 12 g). The maximum relative discrepancy was 5.1% and no consistent trend with muscle length was observed. Nevertheless, the rate of heat loss was always determined under a pre-load of 1 g.

Heat calibration. Calibration was achieved by discharging a capacitor through the electrodes into the muscle, as previously described (Gibbs and Gibson 1969). Experiments were again conducted both on agar "artificial muscles" and on 4 papillary muscles at both temperatures and at the aforementioned two muscle lengths (pre-loads) to ascertain the temperature- and length-dependence of the heat calibration procedure. Once again no consistent trend was evident and the maximum discrepancy observed corresponded to 5.6% of the calibration signal. Nevertheless, calibration was always performed with the muscle under a 1 g pre-load. Given a signal of S (mV), the rate of resting heat production, $\dot{H}_r$ (mW/g), was calculated by

$$\dot{H}_r = S \cdot \frac{H}{C} \cdot \frac{h}{w} \times 10^{-3}$$

where C (mV) is the output of the thermopile in response to a heat calibration step of H (mJ), h (s^{-1}) is the rate of heat loss from the muscle thermopile system, and w (mg) is the weight of the muscle.

Statistical design. A repeated-measures factorial design was developed in which each of eight muscles was subjected to each level of all four treatment factors. The three 2-level

Table 2. Experimental design

Muscle number	Stretch sequence	20° C				27° C			
		With amino acids		Without amino acids		Without amino acids		With amino acids	
		45%	95%	95%	45%	95%	45%	45%	95%
8	d	1	2	3	4	5	6	7	8
1	i	2	1	4	3	6	5	8	7
5	d	3	4	1	2	7	8	5	6
4	i	4	3	2	1	8	7	6	5
3	i	5	6	7	8	1	2	3	4
6	d	6	5	8	7	2	1	4	3
2	i	7	8	5	6	3	4	1	2
7	d	8	7	6	5	4	3	2	1

8 × 8 Latin Square for order of presentation of three conditions; temperature, presence or absence of amino acids, and oxygen fraction. Muscles were randomly assigned to rows as shown in column 1. Stretch sequence indicates whether the loads were presented in increasing (i) or decreasing (d) order: i = 1, 2, 4, 8, 12 and 1 g; d = 1, 12, 8, 4, 2 and 1 g. Entries within the body of the table indicate the order in which the treatments were applied; each sequence of six loads was applied within every treatment

factors (temperature, 20° C and 27° C; oxygen fraction or FO_2, 45% and 95%; and amino acids, presence or absence) were presented to muscles in an order which was dictated by an 8 × 8 Latin Square (Table 2). Within each treatment level-time combination, all six levels of the final factor (pre-load: 1, 2, 4, 8, 12 and 1 g) were presented to each muscle in either the given (ascending) sequence or in mirror-image (descending) sequence. The repeated-measures nature of this design allowed separation of differences among muscles (itself of little physiological interest) from the effects of the treatments, thereby increasing statistical power. Use of a Latin Square, within which the three two-level factors were nested, allowed separation of the effects of those three factors from the effect of the order in which they were presented. As a result the effect of time upon the rate of resting heat production was quantified independently of the other factors.

Data were analysed by Analysis of Variance using the ANOVA procedure supplied as part of the SAS statistical software package (Ray 1982). Null hypotheses were examined for statistical significance, at the 95% level of confidence ($\alpha = 0.05$), using the methods of Rodger (1975).

Experimental protocol. Within the constraints imposed by the statistical design, a protocol was selected which minimized the duration of experiments. From Table 2 it can be seen that temperature was changed only once during the day, i.e. all 24 FO_2-amino acid-pre-load treatment combinations were done at one temperature and then repeated (in a different order) at the other temperature. This reduced the duration of an experiment since about 1 h was required for full thermal stabilization at either temperature. Likewise the Krebs solution (with or without amino acids) was changed only 3 times per day, i.e. all 12 FO_2-pre-load treatment combinations were performed at any given amino acid-temperature combination before changing the Krebs solution. This also reduced experimental duration since changing the Krebs solution required raising the muscle chamber out of the water bath briefly – a procedure which disturbed the baseline output of the thermopile for about 10–15 min. During each of the 8 measurement periods all 6 pre-loads were applied. The muscle was thus out of solution (i.e., muscle chamber drained) for about 15–20 min. At the completion of a measurement period the solution was raised and the muscle allowed to recover for 30 min. The first measurements were made 1 h after cardiectomy and succeeding measurement periods followed at 45–50 min intervals except that somewhat longer was required when bath temperature was changed. The total duration of an experiment was thus about 7 h. During all equilibration and recovery periods the muscle was stimulated isotonically at 1/6 Hz under a 1 g pre-load.

It might be argued that an experimental protocol involving long periods of rest alternating with periods of mild (isotonic) activity could lead to a reduced rate of resting heat production. A preliminary study was undertaken on eight rat papillary muscles to examine this question. Four muscles, under a 1 g pre-load, remained unstimulated throughout the day. The other four muscles contracted isotonically, at a rate of 1/6 Hz, under a 1 g pre-load. Resting heat was measured under this pre-load once every hour for 7 h. These experiments were performed in the absence of amino acids at 27° C in 95% O_2.

Results

The preliminary study yielded a null result. There was no difference in the rate of resting heat production between muscles which were unstimulated and those which were stimulated between measurement periods (Fig. 2). This is in agreement with the findings of Lochner et al. (1968) who showed that, by the fortieth minute of arrest, the oxygen consumption of perfused rat hearts was independent of the workload prior to arrest. Although the rate of resting heat production of all muscles showed the characteristic exponential decline with time, there was no evidence of a difference in either the rate of decline or the mean value over any time interval between muscles which were stimulated continually and those which were quiescent throughout the day. This result (Fig. 2) justifies the experimental protocol used in the main study in which the first measurements were made after an equilibration period (less than an hour) which was probably too brief to allow muscles to reach peak *mech-*

anical performance. The results which follow refer to the main study only.

Figure 3 shows original records from two different muscles under selected treatment conditions. These records were chosen to show the inter-muscle variability in the response of the rate of resting heat production to stretch. Some muscles responded with little change, others with a large increase in resting heat rate. Complete data for one muscle are shown in Fig. 4. Note the extreme stability of the resting heat rate under a 1 g pre-load over each measurement period.

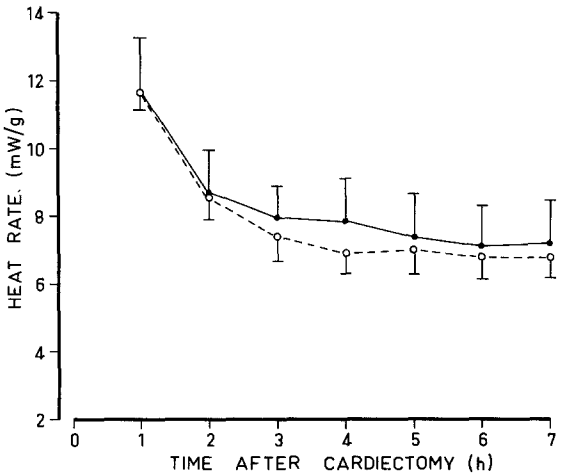

Fig. 2. Results of preliminary study. No difference in rate of resting heat production of rat left-ventricular papillary muscles whether unstimulated (*broken line*) or stimulated continually (*solid line*) between measurement periods. *Vertical bars* are standard errors of the means of $n = 4$ hearts. Between measurement periods the stimulated muscles contracted isotonically against a 1 g pre-load at 1/6 Hz. As measured at the fourth hour (see Methods) there was no difference in maximal active force development between stimulated and unstimulated muscles

Table 3 summarizes the results of the Analysis of Variance of the resting heat data. Note that over 40% of the total variance was due to differences among muscles – a component of no further interest except to underscore the large inter-muscle variability. Because of the large number (12) of interaction terms (for example: temperature-$\times FO_2 \times$ amino acids $\times$ pre-load), and because none proved to be statistically significant, they have been omitted from the Table. All the main effects except amino acids (i.e. temperature, FO_2, stretch or pre-load, and time) were statistically significant (Fig. 5).

Increasing the temperature from 20° C to 27° C increased the cardiac basal metabolic rate by 20% (4.5 to 5.5 mW/g) which corresponds to a Q_{10} of 1.31. Reducing the oxygen fraction from 0.95 to 0.45 atm caused a 25% reduction in heat rate. Lengthening the muscle by the application of pre-loads of greater than 1 g increased the basal metabolic rate. Under the largest (12 g) pre-load the increase ranged from 2% to 65% (mean = 20%). The order in which the pre-loads were applied (i.e., ascending or descending sequence) was not significant. The presence of amino acids failed to elevate the basal rate of heat production; the 7% increase (from 4.8 to 5.2 mW/g) was not statistically significant. As in the pilot study (Fig. 2), the rate of resting heat production fell significantly over the day throughout the first three or

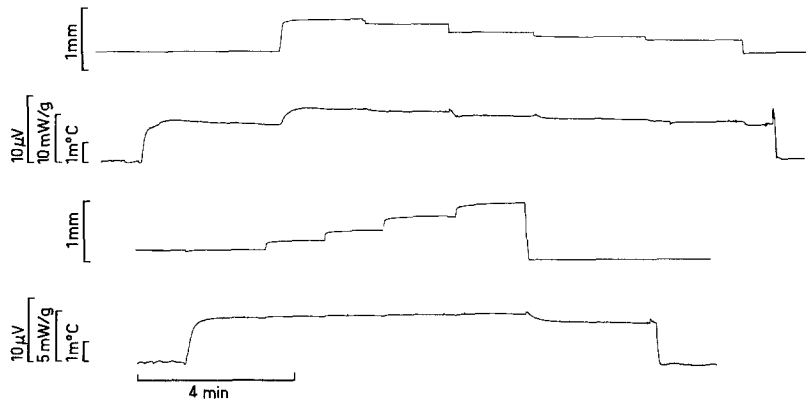

Fig. 3
Original records of a muscle (8, Table 1) with a large (*upper panel*) and a muscle (1, Table 1) with a small (*lower panel*) stretch effect. In each panel the lower trace is the heat output (given in units of temperature change of the active junctions of the thermopile (m° C), thermopile output (μV), and rate of muscle heat production (mW/g) in response to passive loads of 1, 2, 4, 8, 12 and 1 g (*lower panel*) or mirror-image sequence (*upper panel*). *Upper trace* of each panel gives the change in muscle length caused by these passive loads. For both records: $T = 27°C$; $FO_2 = 95\%$; without amino acids

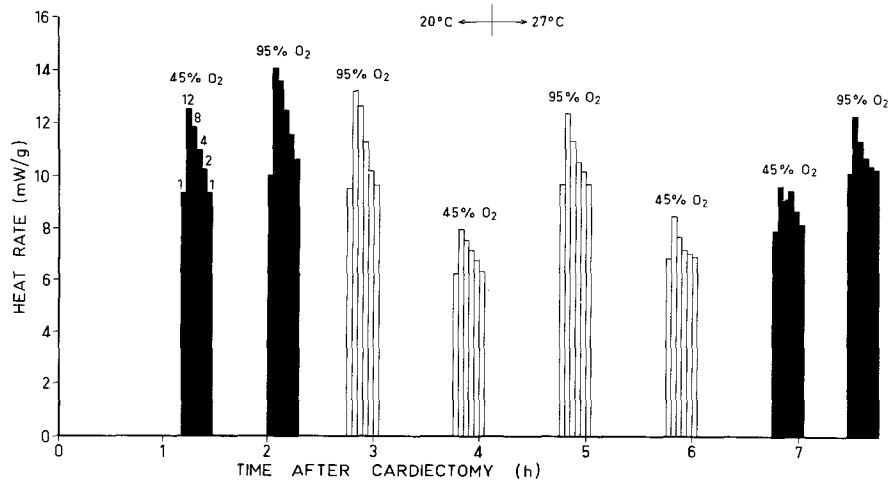

Fig. 4
Complete data for a single rat papillary muscle (8: Table 1). Descending load sequence used throughout (shown for first measurement period only). *Shaded bars*: amino acids present. Temperature: 20° C preceded 27° C. This muscle showed a consistently large stretch effect

four of the eight measurement periods. These data are shown in Fig. 5 as if the periods had been equally spaced although, as described in the Methods, the intervals ranged from 45 to 60 min. It is to be emphasized that the Latin Square nature of the experimental design (see Methods) allows the effect of time to be examined completely independent of the effects of amino acids, temperature, FO_2 and stretch.

The absence of statistically significant interaction effects in the Analysis of Variance (Table 3) is of importance. This means, for example, that the effect of stretch or pre-load upon the basal heat production was the same at both temperatures and both oxygen fractions and did not depend on the presence of amino acids. Likewise the effect of oxygen fraction was independent of temperature, amino acids or muscle length and furthermore, from the absence of significant higher-order interactions, independent of any combination of these factors. It is clear that such a factorial design allows more comprehensive conclusions to be drawn than if a number of separate experiments, each examining the influence of only a single factor, had been performed.

Table 3. Summary of analysis of variance

Source of variation	SS	*df*	MS	*F*
Total	2,035.25	383	–	–
Muscles	872.52	7	–	–
Treatments	792.13	14	–	–
Temperature	86.04	1	86.04	15.13[a]
Amino acids	10.92	1	10.92	1.92
FO_2	188.26	1	188.26	33.12[a]
Time	495.36	7	70.76	12.45[a]
Error (Latin square)	238.77	42	5.68	–
Treatments	85.93	75	–	–
Pre-load	66.85	5	13.37	71.38[a]
Error (factorial)	45.89	245	0.19	–

SS, sum of squares; *df*, number of degrees of freedom; MS, mean square; *F*, variance ratio. Note that Muscles + Treatments + Error = Total for both SS and *df*. (In upper part of Table *df* are 7 + 14 + 42 = 63 as required for an 8 × 8 Latin square.) Only main effects are shown since no interaction effects were significant

[a] Significant at the 95% confidence level ($\alpha = 0.05$)

Discussion

The aim of the present set of experiments was to explore both the direct and interactive effects of four agents: FO_2, stretch, amino acids and temperature in a species (rat) known to have an inherently high rate of both cardiac basal metabolism (Loiselle and Gibbs 1979) and cardiac protein turnover (Earl et al. 1978). The rate of resting heat production of rat left ventricular papillary muscles was determined myothermically in the presence of an exogenous substrate (10 mmol · l^{-1} pyruvate) known to enhance the cardiac basal metabolic rate (Chapman and Gibbs 1974). Although the myothermic method was essentially that which has been in use in the laboratory of Dr. Gibbs over the past decade (for reviews see Gibbs 1978, 1982; Gibbs and Chapman 1979a, b; Chapman 1983), a number of minor technical modifications were made. These aimed to reduce the possibility of thermal artifacts associated with temperature equilibration, oxygenation and humidification of the muscle-thermophile chamber. It must be emphasized that these precautions led to no disparity between the current results and those previously published from this laboratory. Thus the results shown in Fig. 5 (resting heat rate versus time) are compatible with those of Loiselle and Gibbs (1979); the approximately 25% higher rate in the current study can be attributed to the use of pyruvate rather than glucose (Chapman and Gibbs 1974). In fact, the variability in rate of resting heat production among different papillary muscles (see component of variance due to muscles in Table 3, and compare Figs. 2 and 5) likely far exceeds any errors of measurement.

FO_2 effect. As can be seen in Fig. 5, the effect of halving the fraction of oxygen (FO_2) in the medium bathing the papillary muscle was modest, albeit statistically significant. Reducing FO_2 from 95% to 45% reversibly reduced the rate of resting heat production by only 25% (5.7 to 4.3 mW/g).

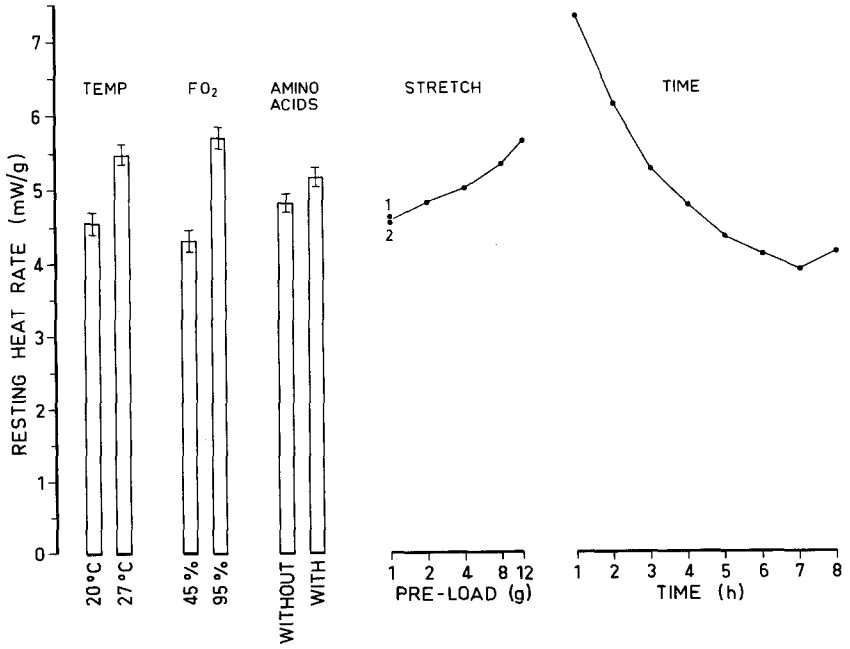

Fig. 5
Main effects (mean ± SE) from Factorial Analysis of Variance (Table 3); all effects except amino acids statistically significant. Stretch effect; 1 and 2 signify initial and final heat rate, respectively, under 1 g pre-load; note logarithmic scale on *abscissa*

A similar result has recently been reported for rabbit papillary muscles in the temperature range 15°C to 30°C (Loiselle and Gibbs 1983). Using rat heart slices, Fuhrman et al. (1950) noted that the rate of oxygen consumption was higher in 100% O_2 than in air. They did not quantitate the effect so it was presumably small and possibly even caused by altered pH. Indeed, Whalen and Fangman (1963) showed that the oxygen consumption of working cat papillary muscles was only halved when the FO_2 was reduced from 98% to 25%. Although lactate production was not measured in the present experiments it is not likely to have made a significant contribution as it is known to account for less than 1% of the basal metabolism of rabbit papillary muscles in vitro (Chapman et al. 1982). It appears that even the high inherent rate of basal oxygen usage by the rat heart is not greatly compromised by the low oxygen *content* of bathing media in vitro.

Amino acid effect. The provision of the amino acids in the bathing medium failed to elevate the rate of heat production of quiescent papillary muscles (Fig. 5). A similar null effect of the addition of amino acids has been reported for the Krebs-perfused dog heart in situ (Gibbs et al. 1980). This result is surprising since both protein synthesis (Millward et al. 1976) and degradation (Ballard 1977) as well as amino acid influx (Baños et al. 1978) are energy-requiring processes, the rate of protein turnover in the heart is high (Earl et al. 1978), cardiac protein synthesis is not diminished by prolonged cardiac arrest (Schreiber et al. 1977), and the provision of amino acids stimulates oxygen utilization in cardiac myocytes in vitro (Burns and Reddy 1978). The concentrations of amino acids used in the current study would have been sufficient to achieve maximal rates of influx in the rat heart (Baños et al. 1978) and insulin, which stimulates amino acid uptake in the myocardium (Wildenthal et al. 1976; for a review see Johnstone 1979), was provided. Likewise the equilibration periods were sufficient to permit maximal accumulation within cardiac cells of most amino acids (Baños et al 1978). If it be assumed that the steady decline with time of the cardiac resting metabolism (Figs. 2 and 5) reflects a slowing of protein metabolism, then such a decline is apparently unaffected by the presence of amino acids.

Stretch effect. The stretch effect was examined in this study not to confirm its existence in yet another species, but expressly to see whether the effect was enhanced by the presence of amino acids. Stretch is known to increase the rate of incorporation of amino acids into both skeletal (Vandenburgh and Kaufman 1981) and cardiac (Lesch et al. 1970; Peterson and Lesch 1972) muscle. It has been suggested (Gibbs 1978; Loiselle and Gibbs 1983) that the stretch effect might be related to a greatly increased rate of protein synthesis such as is seen within 24 h of acute pressure-overload hypertrophy (Everett et al. 1979).

If this suggestion were indeed correct then the stretch-induced increment in resting heat rate should have been increased in the presence of amino acids. This would have resulted in a statistically significant stretch × amino acids interaction term in the Analysis of Variance. Such a result did not occur. Whereas this result offers no support for the stretch-protein synthesis suggestion, neither does it convincingly negate it. Although it seems unlikely (see Amino acid effect, above), it is possible that the amino acids were supplied in a form which rendered them unavailable to the cells. It is possible that the stretch effect indeed reflects protein synthesis but from endogenous supplies of amino acids only.

On the other hand the stretch-induced increment in basal metabolism may be unrelated to protein synthesis. That is, the stretch-induced increase in the uptake of amino acids (Lesch et al. 1970; Peterson and Lesch 1972) may be achieved at little or no nett energetic cost. In this regard, a recent report (Morgan et al. 1983) has shown that the increase in protein synthesis of the perfused rat heart, secondary to an elevated perfusion pressure, is achieved without an increase in oxygen consumption when the heart is arrested. Thus, in the basal state, stretch-induced protein synthesis may be energetically neutral. Such a proposal would likely be best tested using controlled stretches of the left ventricle of isolated, perfused hearts in the arrested state. In the meantime it seems that the stretch effect (which is completely reversible, as demonstrated by the absence of an effect of order of presentation of loads, Fig. 5 and Table 3) must, like the resting cardiac metabolism per se, remain unexplained at present.

Time effect. As can be seen in Figs. 2 and 5, the rate of resting heat production fell progressively with time following cardiectomy to reach a plateau value after some 3 to 5 h. This phenomenon, though also ill-understood, has been previously reported in both isolated papillary muscles (Penpargkul and Scheuer 1969; Chapman and Gibbs 1974; Loiselle and Gibbs 1979) and isolated perfused hearts (Lochner et al. 1968). Such an effect does not occur during cardiac arrest in situ (Gibbs et al. 1980) and seems more pronounced in isolated papillary muscle preparations, using either myothermic or polarographic methods, than in the Langendorff-perfused whole-heart preparation (Gibbs and Kotsanas 1983). As has been previously emphasized (Loiselle and Gibbs 1979), the decline does not coincide with any decrement in mechanical performance of papillary muscle; in fact it is most rapid when the mechanical performance of the muscle is still improving. The time-dependent decline is not likely related to rates of protein synthesis since these are unaffected by three hours of cardiac arrest (Schreiber et al. 1977) and the decline persists despite the provision of amino acids (Table 3). The progressive decline may nevertheless reflect the absence, in Krebs-bathed preparations, of some preferred substrate of cardiac basal metabolism. It is not likely associated with washout of endogenous catecholamines during these lengthy experiments since the provision of exogenous catecholamines in vitro has no effect on cardiac basal metabolism (Coleman et al. 1971; Chapman et al. 1977).

Temperature effect. The precautions detailed in the Methods were made in order to reduce the likelihood of temperature-dependent errors in the measured rate of resting heat production. They were deemed necessary because of the possibility that the recently-reported low Q_{10} value for rabbit cardiac basal heat production (Loiselle and Gibbs 1983) reflected some temperature-dependent error associated with the oxygenation procedure. The equally low Q_{10} value (1.3) reported in the present study (Fig. 5) renders that explanation unlikely. Rather it appears that the temperature coefficient, or Q_{10}, of cardiac basal metabolism in vitro is only obout one-half of the value commonly associated with metabolic processes. Although a companion paper (Loiselle

1985) brings additional experimental evidence to bear on this statement, together with a much fuller review of the literature, it is relevant to point out that Lochner et al. (1968) reported a similarly low Q_{10} value for the K^+ arrested guinea-pig heart. It thus appears that a low effect of temperature must be added to the lengthy list of unresolved problems concerning the nature of cardiac basal metabolism.

Acknowledgements. This research was supported by a Grant-in-Aid and a Research Fellowship from the National Heart Foundation of Australia. It is a pleasure to acknowledge the secretarial assistance of Miss Debra Allen in the preparation of the manuscript.

References

Ballard FJ (1977) Intracellular protein degradation. Essays Biochem 13:1–37

Baños G, Daniel PM, Moorhouse SR, Pratt OE, Wilson PA (1978) The influx of amino acids into the heart of the rat. J Physiol 280:471–486

Burns AH, Reddy WJ (1978) Amino acid stimulation of oxygen and substrate utilization by cardiac myocytes. Am J Physiol 235:E461–E466

Chapman JB (1983) Heat production. In: Drake-Holland AJ, Noble MIM (eds) Cardiac metabolism. Wiley, Chichester

Chapman JB, Gibbs CL (1974) The effect of metabolic substrate on mechanical activity and heat production in papillary muscle. Cardiovasc Res 8:656–667

Chapman JB, Gibbs CL, Loiselle DS (1977) Simultaneous heat and fluorescence changes in cardiac muscle at high rates of energy expenditure: effect of caffeine and isoprenaline. J Mol Cell Cardiol 9:715–732

Chapman JB, Gibbs CL, Loiselle DS (1982) Myothermic, polarographic and fluorometric data from mammalian muscles: correlations and an approach to a biochemical synthesis. Fed Proc 41:176–184

Coleman HN, Sonnenblick EH, Braunwald E (1971) Mechanism of norepinephrine-induced stimulation of myocardial oxygen consumption. Am J Physiol 221:778–783

Cranefield PF, Greenspan K (1960) The rate of oxygen consumption of quiescent cardiac muscle. J Gen Physiol 44:235–249

Delbridge LM, Loiselle DS (1981) An ultrastructural investigation into the size dependency of contractility of isolated cardiac muscle. Cardiovasc Res 15:21–27

Earl CA, Laurent GJ, Everett AW, Bonnin CM, Sparrow MP (1978) Turnover rates of muscle protein in cardiac and skeletal muscles of dog, fowl, rat and mouse: turnover rate related to muscle function. Aust J Exp Biol Med 56:265–277

Everett AW, Sparrow MP, Taylor RR (1979) Early changes in myocardial protein synthesis in vivo in response to right ventricular pressure overload in the dog. J Mol Cell Cardiol 11:1253–1263

Fuhrman GJ, Fuhrman FA, Field J (1950) Metabolism of rat heart slices, with special reference to effects of temperature and anoxia. Am J Physiol 163:642–647

Gibbs GL (1978) Cardiac energetics. Physiol Rev 58:174–254

Gibbs CL (1982) Modification of the physiological determinants of cardiac energy expenditure by pharmacological agents. Pharmacol Ther 18:133–157

Gibbs CL, Chapman JB (1979a) Cardiac heat production. Annu Rev Physiol 41:507–519

Gibbs CL, Chapman JB (1979b) Cardiac energetics. In: Berne RM, Sperelakis N (eds) The cardiovascular system. Handbook of physiology, ch 22. Am Physiol Soc, Bethesda MD, pp 775–804

Gibbs CL, Gibson WR (1969) Effect of ouabain on the energy output of rabbit cardiac muscle. Circ Res 24:951–967

Gibbs CL, Gibson WR (1972) Isoprenaline, propranolol and the energy output of rabbit cardiac muscle. Cardiovasc Res 6:508–515

Gibbs CL, Kotsanas G (1983) The metabolism of quiescent rabbit myocardium. Proc Aust Physiol Pharmacol Soc 14:18

Gibbs CL, Mommaerts WFMH, Ricchiuti NV (1967) Energetics of cardiac contractions. J Physiol 191:25–46

Gibbs CL, Papadoyannis DE, Drake AJ, Noble MIM (1980) Oxygen consumption of the nonworking and potassium chloride-arrested dog heart. Circ Res 47:408–417

Greenspan K, Cranefield PF (1963) Influence of some factors on oxygen uptake of canine cardiac Purkinje fibres. Am J Physiol 24:5–8

Hill AV (1928) The diffusion of oxygen and lactic acid through tissues. Proc Roy Soc London Series B 104:39–96

Hjalmarson A, Isaksson O (1972) In vitro work load and rat heart metabolism. I. Effect on protein synthesis. Acta Physiol Scand 86:126–144

Johnstone RM (1979) Electrogenic amino acid transport. Can J Physiol Pharmacol 57:1–15

Lee KS (1960) The relationship of the oxygen consumption to the contraction of the cat papillary muscle. J Physiol 151:186–201

Lesch M, Gorlin R, Sonnenblick EH (1970) Myocardial amino acid transport in the isolated rabbit right ventricular papillary muscle. Circ Res 27:445–460

Lochner W, Arnold G, Muller-Ruchholtz ER (1968) Metabolism of the artificially arrested heart and of the gas-perfused heart. Am J Cardiol 22:299–311

Loiselle DS (1982) Stretch-induced increase in resting metabolism of isolated papillary muscle. Biophys J 38:185–195

Loiselle DS (1985) The effect of temperature on the basal metabolism of cardiac muscle. Pflügers Arch 405:163–169

Loiselle DS, Gibbs CL (1979) Species differences in cardiac energetics. Am J Physiol 237:H90–H98

Loiselle DS, Gibbs CL (1983) Factors affecting the metabolism of resting rabbit papillary muscle. Pflügers Arch 396:285–291

Millward DJ, Garlick PJ, James WPT, Sender P, Waterlow JC (1976) Protein turnover. In: Cole DJA, Boorman KN, Buttery PJ, Lewis D, Neale RJ, Swan H (eds) Protein metabolism and nutrition; proceedings. Butterworths, London, pp 49–69

Morgan HE, Kira Y, Gordon EE, Kochel PJ (1983) Mechanical factors determining rates of protein synthesis and degradation. J Mol Cell Cardiol, suppl 2 15:19

Page E, McCallister LP, Power B (1971) Stereological measurement of cardiac ultrastructure implicated in excitation-contraction coupling. Proc Natl Acad Sci USA 68:1465–1466

Penpargkul S, Scheuer J (1969) Metabolic comparisons between hearts arrested by calcium deprivation or potassium excess. Am J Physiol 217:1405–1412

Peterson MB, Lesch M (1972) Protein synthesis and amino acid transport in the isolated rabbit right ventricular papillary muscle. Circ Res 31:317–327

Pool PE, Sonnenblick EW (1967) The mechanochemistry of cardiac muscle. I. The isometric contraction. J Gen Physiol 50:951–965

Ray AA (1982) (ed) SAS user's guide: Statistics. SAS Institute Inc, Cary, NC

Rodger RS (1975) The number of non-zero, post hoc contrasts from ANOVA and error rate I. Br J Math Stat Psychol 28:71–78

Schreiber SS, Hearse DJ, Oratz M, Rothschild MA (1977) Protein synthesis in prolonged cardiac arrest. J Mol Cell Cardiol 9:87–100

Vandenburgh HV, Kaufman S (1981) Stretch-induced growth of skeletal myotubes correlates with activation of the sodium pump. J Cell Physiol 109:205–214

Whalen WJ (1960) Some factors influencing O_2 consumption of isolated cardiac muscle. Am J Physiol 198:1153–1156

Whalen WJ, Fangman J (1963) Respiration of heart muscle as affected by oxygen tension. Science 141:274–275

Wildenthal K, Griffin EE, Ingwall JS (1976) Hormonal control of cardiac protein and amino acid balance. Circ Res suppl 1, 38:I138–I142

Received March 1, 1984/Accepted June 4, 1985