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HEAT PRODUCTION OF QUIESCENT VENTRICULAR TRABECULAE ISOLATED FROM GUINEA-PIG HEART

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SUMMARY

1. A new calorimetric technique has been developed which allows continuous measurement of the rate of energy expenditure in superfused preparations of cardiac muscle. Thin trabeculae of guinea-pig ventricular muscle were mounted in a Perspex tube of 0·8 mm inner diameter and the temperature difference of the perfusate upstream and downstream of the preparation was measured.

2. The resting heat rate of trabeculae of 240–575 μm diameter from guinea-pig heart was determined repeatedly for up to 6 h after cardiectomy. It did not vary with time during the course of the experiment.

3. The average resting heat rate measured in HEPES-buffered Tyrode solution containing 20 mM-glucose and 2 mM-pyruvate as substrates was 130 ± 29 mW/g dry weight or 36 ± 8 mW/cm^3 of tissue ($n = 15$). This is an order of magnitude larger than the resting heat rate reported in the literature for isolated cardiac preparations.

4. After omitting the pyruvate from the superfusate the resting heat rate decreased to 60–70 % of its steady value within 4 min. After readmission of pyruvate this effect was reversed. The average resting heat rate with glucose as sole substrate was 23 ± 4 mW/cm^3.

5. Uncoupling of the mitochondria by 50 μM-2,4-dinitrophenol (DNP) increased the heat rate up to 170 mW/cm^3. This effect could be maintained for several minutes and was fully reversible. Raising the external K^+ concentration to 150 mM (NaCl replaced by KCl) induced a transient rise in the rate of heat production up to 115 mW/cm^3.

6. The heat production during uncoupling of the mitochondria and during potassium contractures was inversely related to the diameter of the preparation. Calculation based on Hill's equation (Hill, 1928) indicated that this was caused by the development of anoxia at the core of the preparation.

7. In contrast, the rate of heat production of quiescent preparations was not correlated with diameter and calculation indicated that at rest there was no anoxic core. The high value of resting heat rate found in the present study is discussed within the context of the large variation of 1·7–25 mW/g reported in the literature for resting metabolic rate of cardiac muscle.

INTRODUCTION

When a mammalian heart is arrested it continues to consume oxygen at a considerable rate. This resting oxygen consumption usually amounts to 20–30% of that of the working heart, but may, according to the literature (cf. Lochner, Arnold & Müller-Ruchholtz, 1968; Loiselle & Gibbs, 1979; Gibbs & Kotsanas, 1986), vary over a much wider range corresponding to energy outputs from 1·7 to 25 mW/g. The precise values depend on temperature, animal species, composition of the perfusate used to arrest the heart, time of measurement after arrest, and the rate of coronary perfusion (Gibbs, 1978).

In trying to elucidate the underlying mechanisms experiments on isolated papillary muscles have been carried out in several laboratories. When resting energy expenditure was measured as the resting heat rate of quiescent papillary muscles mounted on thermopiles it was found for various mammalian species that it was not constant in time but decayed for 3–4 h after cardiectomy to about 50% of the value measured initially. The values found during the final steady state (2·0–6·2 mW/g; Loiselle & Gibbs, 1979) were in reasonable agreement with oxygen consumption values usually encountered in arrested perfused hearts (Gibbs, Papadoyannis, Drake & Noble, 1980; Gibbs & Kotsanas, 1986). However, when resting energy expenditure was measured as oxygen consumption of papillary muscle the values obtained were usually at least twofold higher (between 3·8 and 22·8 mW/g; Lee, 1960; Whalen, 1960; Cranefield & Greenspan, 1960; Gibbs, Woolley, Kotsanas & Gibson, 1984).

The main difference between these two types of measurement of resting energy expenditure is that with the measurement of oxygen consumption the muscles are continuously superfused while in the thermopile experiments the preparations are out of solution during the measurement period, but it is not clear if this is the reason for the discrepancies. In order to get a better understanding of the factors controlling resting metabolism we decided to measure the resting heat rate of thin trabeculae of guinea-pig ventricular muscle with a new technique in which the preparation is continuously superfused. Some of the results have already been reported in preliminary form (Buitenweg, Daut & Elzinga, 1987).

METHODS

Dissection and solutions

Guinea-pigs weighing 200–250 g were killed by a sharp blow on the head and the heart was quickly excised. The initial part of the dissection was carried out in a solution (A) which contained (mM): NaCl, 90; potassium glutamate, 22·5; sodium pyruvate, 7·5; $CaCl_2$, 2; $MgSO_4$, 3; Na_2HPO_4, 0·5; glucose, 20; taurine, 30; creatine, 10; HEPES, 10. The solution was gassed with oxygen, the temperature was 30 °C and the pH was adjusted to 7·4 with Tris. When a suitable trabecula had been found the heart was exposed to a similar solution (B) which contained 90 mM-potassium glutamate and 22·5 mM-NaCl but was otherwise identical to solution A. After a few minutes the ends of the trabecula were tied off with a fine nylon thread and the preparation was cut out and transferred to a 'recovery chamber' made of stainless steel, which was connected with the actual recording chamber. The recovery chamber contained solution A and was heated to 37 °C. The preparation was allowed to recover for about 10 min, then solution A was exchanged for a modified Tyrode solution (C), which was used for the heat measurements. It contained (mM): NaCl, 147; KCl, 3; $CaCl_2$, 2; $MgSO_4$, 1; Na_2HPO_4, 1; sodium pyruvate, 2; glucose, 20; HEPES, 10. The pH

was adjusted to 7·4 by the addition of Tris. The solution was continuously bubbled with oxygen and heated to 37 °C. In some experiments the preparations were exposed to a high-K^+ solution (150 mM-K^+). This was identical to solution C except that all of the NaCl was replaced by KCl.

Recording chamber

The diameter and length of the preparation between the two knots were measured under the microscope at 40× magnification. Excitability was checked by electrical stimulation. If the trabecula showed normal contractile behaviour, i.e. rapid and strong twitches as judged by observation under the microscope, the nylon threads at either end were tied to the ends of two Polythene tubes of 0·5 mm diameter connected to micrometer screws. By turning the two micrometer screws simultaneously the preparation was then moved into the actual recording chamber which consisted of a Perspex tube of 0·8 mm i.d. and 1 mm o.d. (Fig. 1*A*).

The inlet and outlet of the tube were glued into a stainless-steel block. The tube was surrounded by air to reduce heat loss. The steel block was mounted in a cylindrical block of aluminium of 10 cm diameter and covered with an aluminium lid. The aluminium block was placed in a circular tank of polyvinylchloride of 15 cm i.d. which was isolated from the environment by a double wall filled with cotton wool. The space between the aluminium block and the wall of the tank contained a spiral of copper tubing and was filled with aluminium powder. The temperature of the tank was kept constant by perfusing the copper tube with water at 37 °C. Water temperature fluctuated by less than 0·01 °C.

The recording chamber was perfused at a constant speed by means of a suction pump (Precision Pump M16, Reichelt Chemie Technik, Heidelberg, F.R.G.). The solution could be changed by switching a four-port valve located about 50 mm on the upstream side of the recording chamber. The two input ports of the four-port valve were connected to the inlets of the tank by stainless-steel tubes (1 mm o.d.) of about 40 cm length each, which were embedded in the aluminium powder surrounding the aluminium block in order to ensure temperature equilibration. The perfusate entering the tank was preheated to approximately 37 °C and equilibrated with 100% oxygen; the P_{O_2} was 740 mmHg as measured with an automatic acid–base laboratory (ABL 330, Radiometer, Copenhagen) at 37 °C. When perfusing the recording chamber at the usual speed the P_{O_2} at the outlet of the tank was found to be 700 mmHg. Thus the P_{O_2} in the actual recording chamber was about 720 mmHg (not 520 mmHg as stated in Buitenweg *et al.* 1987).

The temperature difference between the upstream and the downstream side of the preparation was measured with 2 × 6 chromel–constantan thermocouples mounted 4 mm apart as indicated schematically in Fig. 1*A*. For simplicity only two thermocouples on either side are shown. They were embedded in the wall of the Perspex tube (100 μm thickness) and had no direct contact with the perfusing solution. The diameter of the wires was 50 μm. The rate of perfusion had to be chosen carefully. With high rates the measured temperature change per microwatt was very small; with very slow rates of perfusion the heat loss across the wall of the tube and the backward diffusion of heat towards the upstream thermocouple became appreciable. The optimal rate of perfusion for measuring resting heat rate with high resolution in our system was 1 μl/s.

The voltage output of each thermocouple was 63·3 μV/°C as determined by transferring identical thermocouples between solutions with different temperatures (32 and 42 °C) and measuring the resulting voltage change. The resistance of the twelve thermocouples in series was 25 Ω. Their voltage output was measured with a low-noise differential amplifier consisting of nine operational amplifiers (ANCOM CM 1251) in parallel (Dijkema, Elzinga & Hollewijn, 1985). The amplification was checked by applying a known voltage difference to the two inputs of the amplifier with the thermocouples in place.

The length of the preparation was set under the microscope by adjusting the two micrometer screws so that the separation of the attachment points was just below that causing the nylon threads to be taut. The muscle preparation was slack at this setting. The friction of the nylon threads (10 μm diameter) at the wall of the tube was very small. The position and excitability of the preparation was checked visually after opening the tank at intervals of 1–2 h during each experiment. The stimulating electrodes consisted of two platinum wires of 50 μm diameter (not shown in Fig. 1*A*). They entered the chamber through two fine holes on either side of the tube (resealed with glue) and extended about 1 mm in the longitudinal direction on the inner side of the wall.

By turning the two micrometer screws simultaneously the preparation could be moved

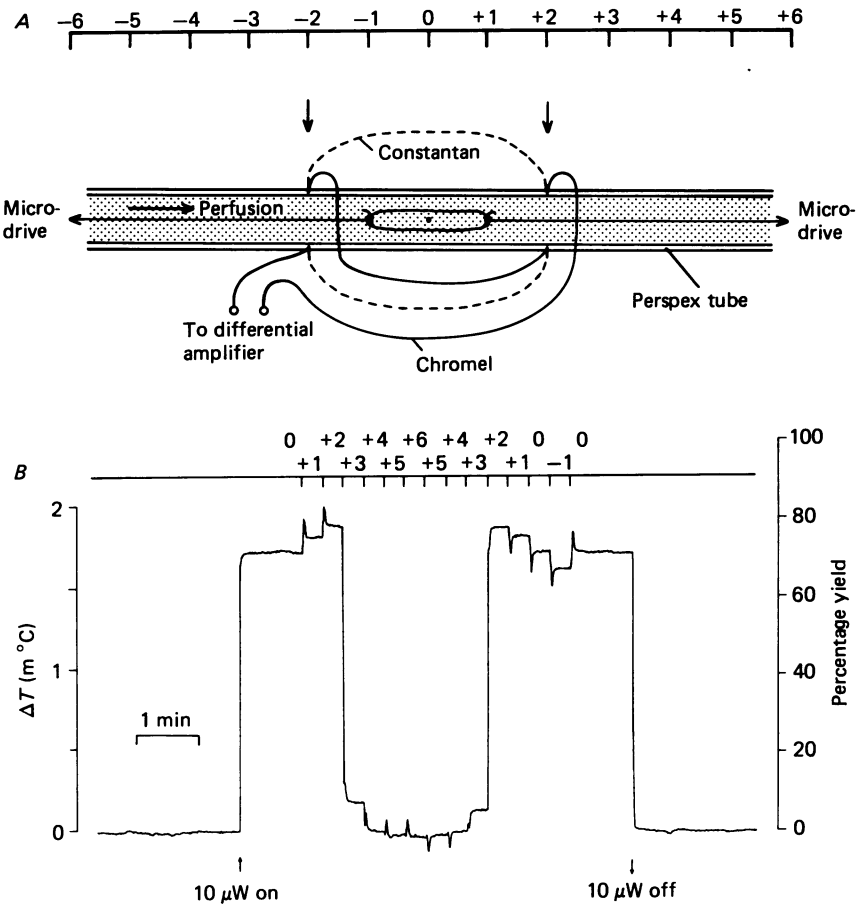

Fig. 1. *A*, schematic diagram of the recording system. The scale gives the spatial co-ordinates in millimetres, position 0 representing the middle of the chamber. Six thermocouples were mounted on each side at +2 and −2 mm from the centre of the chamber; only two of them are shown for clarity. The location of the thermistor, or of the mid-point of the preparation, at position 0 is marked by a dot. *B*, calibration of the recording system with a small thermistor bead. Initially the thermistor was at position 0 and the 10 μW power was switched on and off at the arrows. The thermistor was moved stepwise every 20 s to the position indicated on the scale. The voltage difference recorded by the differential amplifier is shown. On the left-hand ordinate this voltage has been converted to m °C using the known properties of the thermocouples. 1 m °C corresponds to 2·63 mV. On the right-hand ordinate the voltage output expected if all the power dissipated by the thermistor was detected by the measuring system is shown as 100%.

longitudinally along the axis of the tube. In the records shown in the Results section the position of the mid-point of the preparation (marked by a dot) on the spatial co-ordinate shown in Fig. 1*A* is indicated. 0 is defined as the position in the middle between the upstream and downstream thermocouples, position +1 indicates that the preparation was moved 1 mm to the right (downstream) and position −1 indicates that the preparation was moved 1 mm to the left (upstream), etc. At the end of the experiment the dead ends of the preparation (about 100 μm) beyond the two knots were cut off carefully with a pair of fine scissors and the dry weight of the live part of the preparation was determined on a micro-balance (Model 29, CAHN, Cerritos, CA,

U.S.A.). Control experiments were carried out to test whether dead myocardial tissue produced any heat. First, resting heat rate was measured the usual way. Then the preparation was pulled out of the recording chamber and crushed with fine forceps. Subsequently the trabecula was pulled back into the recording chamber. It was found that in the crushed preparations resting heat production was abolished.

Statistical analysis

All the results are expressed as mean ± standard deviation. The number of preparations (n) from which the data were obtained is indicated in parentheses.

RESULTS

Calibration of the recording system

The spatial and temporal characteristics of heat transfer to the solution and to the thermocouples were studied by introducing a thermistor bead of 0·4 mm diameter in the recording chamber and dissipating a known amount of power. The chamber was continuously perfused with Tyrode solution at a rate of 1 μl/s corresponding to a velocity of about 2 mm/s. Initially the thermistor bead was at position 0, i.e. in the middle of the chamber. Figure 1*B* shows that when 10 μW was dissipated through the thermistor bead by applying a constant voltage (arrow), the potential recorded at the input of the differential amplifier changed in the positive direction indicating that the downstream thermocouples became relatively hotter than the upstream thermocouples.

The thermistor was then moved 1 mm to the right (downstream) every 20 s until it reached position +6. It can be seen that at positions +1 and +2 there was a slight increase in the temperature of the downstream thermocouple. When the thermistor was moved from position +2 to position +3, i.e. when it passed the downstream thermocouple, there was a large decrease in temperature difference. The voltage output recorded at positions 4–6 during the 10 μW power input was exactly the same as that recorded at position 0 with no power applied (vertical arrows). This indicates that the heat produced by the thermistor at position +4, +5 or +6 had no effect on the temperature difference between the two sets of thermocouples. The small temperature change recorded upon moving the thermistor from position +3 to +4 and vice versa suggests that only a small fraction of the heat given off to the solution by the thermistor was conducted backwards to the downstream thermocouples which were located exactly at position +2.

The left-hand ordinate gives the temperature difference calculated from the measured voltage change and the known sensitivity of the thermocouples ($6 \times 63{\cdot}3$ μV/°C, see Methods). The temperature change (ΔT) expected if no heat is lost to the environment can be calculated from the equation

$$\Delta T = Vi\, C_{\mathrm{h}}^{-1} f^{-1}, \tag{1}$$

where V is voltage (V), i is current (A), (Vi) is the power (W) dissipated in the thermistor (corresponding to the rate of heat production by the preparation), C_{h} is the heat capacity of Tyrode (J °C^{-1} cm^{-3}), and f is the rate of perfusion (cm^3 s^{-1}).

The temperature change calculated from eqn (1) is 2·39 m °C. This value corresponds to 100 % on the right-hand ordinate. It can be seen in Fig. 1*B* that at

position 0, 72% of the applied heat is recorded and only 28% is lost to the environment. Between position −1 and +1 the yield of the system varied almost linearly between 67 and 76%. This indicates that the space constant of the heat loss in the longitudinal direction was large compared to the 4 mm distance between the upstream and downstream thermocouples. Based on these observations the heat production of small trabeculae was measured by positioning their mid-point at

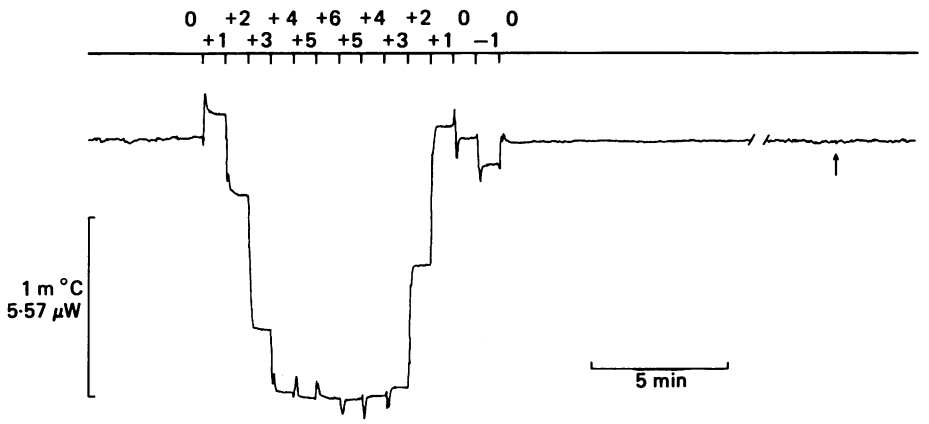

Fig. 2. Typical measurement of the resting heat rate of a trabecula of 0·375 mm diameter and 1·750 mm length. The preparation was moved by turning the two micrometer screws connected to the ends of the preparation. The position of the mid-point of the preparation on the spatial co-ordinate of Fig. 1*A* is given on top. At the arrow on the right-hand part of the record the four-port valve located 50 mm upstream of the preparation was switched to effect a change between two solutions of identical composition.

position 0 and assuming that then on average 72% of the heat given off to the solution was recorded. It should be noted that the absolute calibration of resting heat rate did not depend on this assumption because the system was calibrated by dissipating a known amount of power. Over the whole range tested (0–100 μW) thermocouple output was strictly proportional to the power dissipated through the thermistor.

An example of a determination of the heat rate produced by a quiescent trabecula is shown in Fig. 2. The preparation was moved by 1 mm every minute. The changes in temperature recorded between positions +2 and +4 were less abrupt than the changes recorded with the thermistor (Fig. 1*B*) because the heat production of the trabecula is distributed over its entire length whereas the thermistor resembles a point source of heat. Moving the preparation from position +4 to +5 and +6 hardly produced any change in voltage output indicating that the entire preparation was downstream of the downstream thermocouples. The slight hysteresis observed at positions +2 and +3 can be explained by the fact that the trailing nylon thread had to be pulled taut before it could move the preparation in the other direction. The right-hand part of the record shows that switching between two solutions of identical composition (arrow) produced no artifacts.

The results presented in Fig. 1 show that the presence of the thermistor *per se* in between the two sets of thermocouples, with no power applied, does not influence the

temperature difference between the two measuring points. However, it might be argued that the presence of a large trabecula in between the thermocouples could cause a permanent change in the voltage output by deflecting the flow of the solution. The results shown in Fig. 3 demonstrate that this is not the case. First (marker *a*) the preparation was moved stepwise from position 0 to position 6 and back to position 0 like in Fig. 2. Subsequently the superfusing solution was

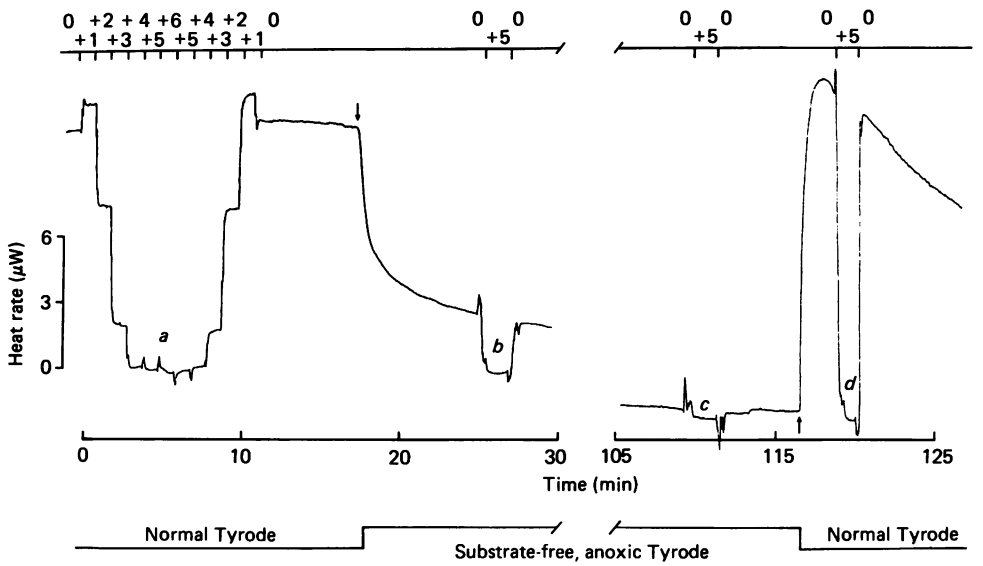

Fig. 3. The resting heat rate of a trabecula of 520 μm diameter before, during and after an exposure to substrate-free, anoxic Tyrode solution lasting about 100 min. Note the gap of 75 min between the two parts of the record (time scale on the bottom). At the beginning of the record a determination of resting heat rate was carried out as in Fig. 2. At the times indicated on the top line the preparation was moved to position +5, i.e. completely out of the measuring zone, and back to position 0.

exchanged for a substrate-free anoxic solution, i.e. sodium pyruvate and glucose were omitted and the P_{O_2} was reduced from 720 to 27 mmHg by equilibrating the solution with nitrogen instead of oxygen. A few seconds after the solution change there was a steep drop in the rate of heat production of the preparation. The delay represents the time needed for the solution to flow from the four-port valve used for the solution change to the preparation.

Seven minutes after the solution change (marker *b*) the preparation was moved to position +5 and then back to position 0. It can be seen that the reference potential with no preparation in between the measuring points was unchanged. This means that the temperature change recorded was fully due to a reduced heat production of the preparation. The preparation was again moved out of the measuring zone (marker *c*) 91 min after the solution change. Note the 75 min time interval between the two parts of the record. At this time (*c*) the heat production of the preparation had decayed almost to zero and hardly any change in the temperature signal was found when the trabecula was moved to position +5 and back. After 109 min the

solution change was reversed, i.e. oxygen and substrates were readmitted. This resulted in a rapid and large rise in heat production. The overshoot in heat rate beyond its resting level was followed by a slow decline to very low values. The preparation was once again (*d*) moved to position +5 and then back to position 0. The baseline was still almost the same as before.

These findings show, firstly, that the presence of the preparation itself in the space between the two measuring points produces no artifact in the voltage output. Thus the voltage change recorded when moving the preparation from the middle of the recording chamber downstream by 5 or 6 mm gives a correct measurement of the rate of heat production of the preparation. Secondly, this experiment shows that the reference potential remained virtually constant over long periods of time. The drift was typically 0·5–1 μW/h. This means that this technique allows continuous recording of the rate of heat production over long periods of time. The speed of the temperature change after readmission of oxygen gives an indication of the temporal resolution of the system. At the perfusion rate used normally (1 μl/s) the response to a rectangular power pulse through the thermistor at position 0 reached 90% of its steady-state level after about 2 s (compare with Fig. 1*B*).

The rate of heat production of quiescent trabeculae

The time dependence of resting heat rate was studied in eleven preparations superfused continuously with modified Tyrode solution containing 20 mM-glucose and 2 mM-pyruvate (solution C, see Methods). Figure 4 shows a plot of resting heat determined as described above against time after cardiectomy, different symbols indicating different preparations. The diameter of the trabeculae ranged from 240 to 575 μm, the mean being 364 ± 88 μm. It can be seen that in individual preparations the resting heat rate remained rather constant for up to 6 h after cardiectomy. Usually the resting heat rate varied by less than 5% between measurements. The mean rate of heat production was 130 ± 29 mW/g dry weight ($n = 15$). This average includes four experiments in which only one determination of resting heat rate was carried out before challenging the preparation with a different solution (see Fig. 5). In fact, in most of the trabeculae the response to different superfusates was tested at later times during the experiment (see below). However, since such interventions sometimes gave rise to long-lasting changes in the metabolic state only heat rates measured prior to any solution change are shown in Fig. 4.

The right-hand ordinate of Fig. 4 indicates the heat rate in mW per cm^3 of tissue, which was obtained by multiplying the left-hand ordinate with the *average* dry weight-to-volume ratio ($0{\cdot}28 \pm 6$ g dry weight/cm^3; $n = 15$). The average resting heat rate per cm^3 of tissue was 36 ± 8 mW. Every 1–2 h the tank was opened to check the position and the excitability of the preparation by inspection under the microscope. All preparations showed rapid twitches upon electrical stimulation. The speed or amplitude of the contraction did not appear to change during the course of the experiment. No after-contractions or periods of spontaneous activity were observed.

In Fig. 5 the average resting heat rate measured in each of the fifteen preparations has been plotted against radius (●). There was no significant correlation between resting heat rate and radius. The curved line represents the critical heat rate for a given radius (at the prevailing P_{O_2} of 720 mmHg) that would just lead to the

development of an anoxic core as calculated from Hill's equation (Hill, 1928, 1965). Points above the line indicate that the core of the preparation was anoxic. It can be seen that, except for the largest preparation, the resting heat rate was always lower than that which would have caused an anoxic core.

It is well known that resting heat production depends on the metabolic substrate (Krebs, 1950; Chapman & Gibbs, 1974; Gibbs & Kotsanas, 1986). In order to facilitate comparison of our measurements with the results of other investigators we

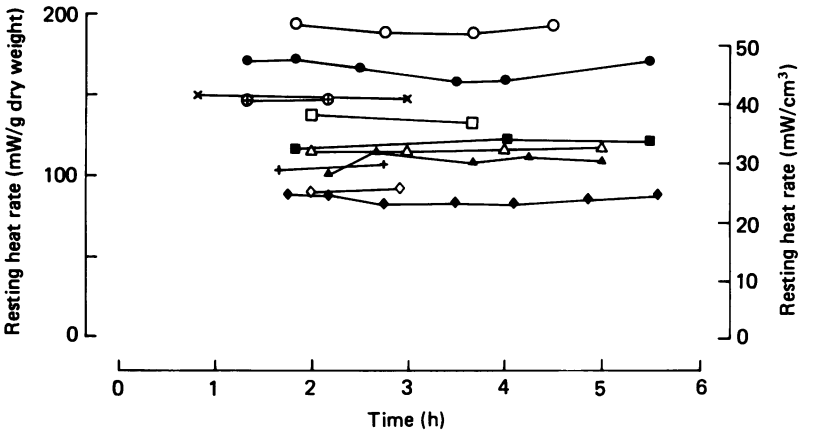

Fig. 4. The time dependence of resting heat rate. The abscissa indicates time after cardiectomy. Different symbols indicate different preparations. Every symbol represents one measurement where the preparation was moved out of the measuring zone and back again. The data are plotted as mW/g dry weight (left). The right-hand scale was obtained by multiplying the left-hand scale with the average dry weight/volume ratio measured in fifteen preparations.

studied the effect of omitting the 2 mM-pyruvate from the standard solution. On the left-hand side of Fig. 6 a determination of resting heat rate in our standard solution containing 20 mM-glucose and 2 mM-pyruvate is shown. After omission of pyruvate the resting heat rate decreased by 35% within 4 min. Subsequently the preparation was again moved stepwise to position +5 and back to verify that the baseline had not changed. After readmission of 2 mM-pyruvate the change in heat rate was reversed within 4 min. The average rate of heat production in modified Tyrode solution containing 20 mM-glucose as sole substrate was found to be 83 ± 11 mW/g dry weight, 23 ± 4 mW/cm^3 ($n = 8$).

The speed of the change in heat rate during omission of pyruvate probably represents (i) the exchange of the solution in the chamber, (ii) the radial diffusion of substrate within the preparation, and (iii) the decay of cytosolic pyruvate concentration due to oxidation in the mitochondria. The heat rate was found to decay with a time constant of 45–70 s after the solution change ($n = 8$).

High-potassium contractures and effects of 2,4-dinitrophenol

The left-hand part of the record shown in Fig. 7 illustrates the effects of replacing all of the NaCl in the superfusing solution by KCl. A rapid rise in the rate of heat

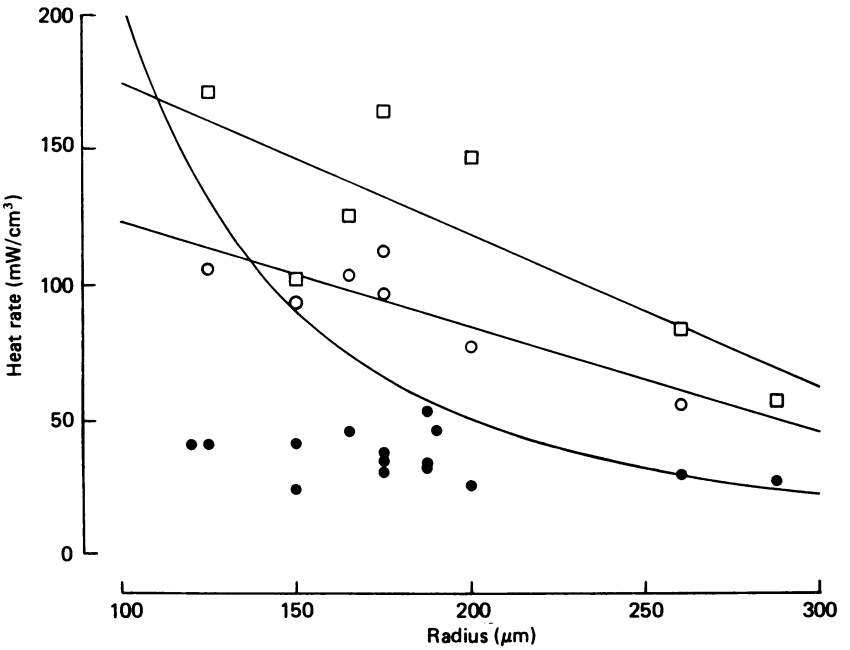

Fig. 5. Dependence of resting heat rate (●), peak heat rate during high-K^+ contracture (○), and uncoupled heat rate during application of 50 μM-2,4-dinitrophenol (□), on the radius of the preparation. Four experiments in which resting heat rate was determined only once before changing the solution were also included (not shown in Fig. 4). Resting heat rate was not significantly correlated with diameter (correlation coefficient −0·36). The straight lines are linear regression lines drawn through the data obtained with DNP (correlation coefficient −0·78) and high external K^+ (correlation coefficient −0·85). The curved line was calculated from Hill's equation (Hill, 1928) using a diffusion constant of O_2 through muscle of $1{\cdot}66 \times 10^{-5}$ cm^2 min^{-1} atm^{-1} at 37 °C (Krogh, 1919; Hill, 1965) and a P_{O_2} of 720 mmHg.

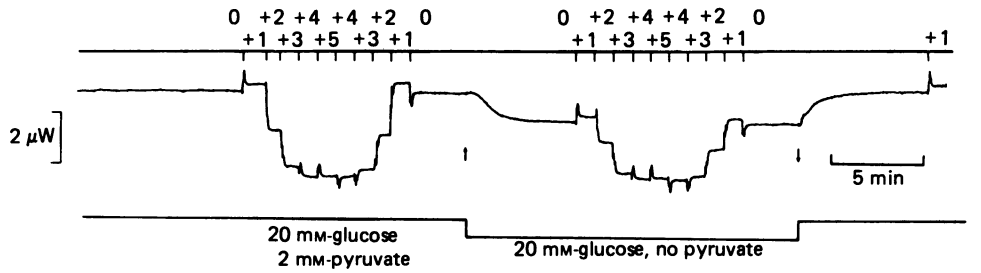

Fig. 6. The effects of changing the substrate on the rate of heat production of a trabecula of 320 μm diameter. After the initial determination of the resting heat rate the standard Tyrode solution containing 20 mM-glucose and 2 mM-pyruvate was switched for 18 min to a solution containing no pyruvate. When the heat rate in the pyruvate-free solution had reached a steady state the determination of resting heat rate was repeated.

production from 5 μW to a peak of about 13 μW was followed by a slow decline to 11 μW. Upon changing back to the normal solution the increase in heat rate was reversed within 3 min. A few minutes later the same procedure was repeated and produced a very similar effect. Note, however, that the heat rate measured after the contracture was somewhat lower than the previous steady-state value. This was found in four out of seven preparations. Observation under the microscope showed that application of 150 mM-external K^+ produced a contracture which was readily reversed after switching back to control solution.

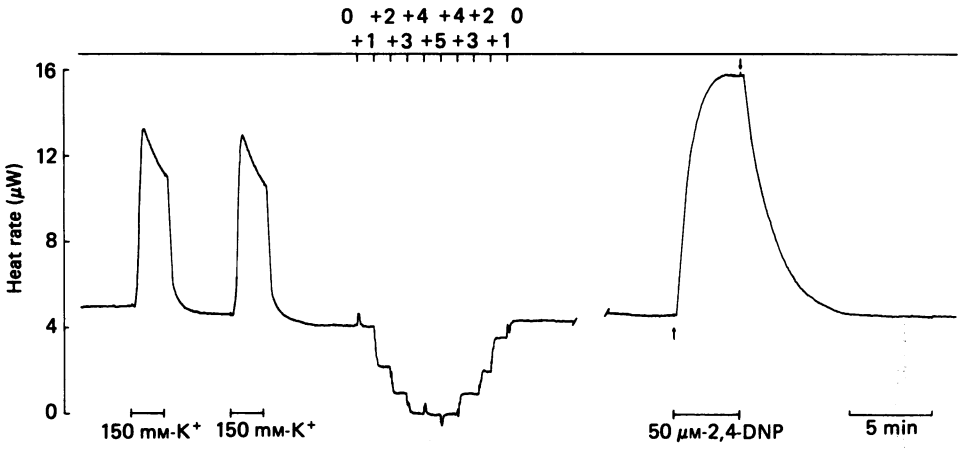

Fig. 7. The effects of a high-K^+ solution (150 mM-KCl, left) and 50 μM-2,4-DNP (right) on the rate of heat production of a preparation of 330 μm diameter. In the middle of the record resting heat rate was determined as in Fig. 2.

In Fig. 5 the peak heat rate per cm^3 of tissue during potassium contractures has been plotted against radius of the preparation (○). Peak heat rate increased with decreasing radius (correlation coefficient −0·85). This is apparently due to the development of an anoxic core caused by the greatly increased oxygen consumption of the peripheral cells. All preparations except the smallest one were above the line representing the critical heat rate calculated from Hill's equation for radial diffusion of oxygen in a cylindrical preparation.

Uncoupling of mitochondria increases the rate of substrate oxidation and should therefore greatly increase the rate of heat production. The right-hand part of Fig. 7 shows a representative experiment in which 50 μM-2,4-dinitrophenol (DNP) was added to the superfusate. The rate of heat production increased from 5 to 16 μW within 3 min. This high heat rate could be maintained for up to 8 min (longest interval tested). After wash-out of DNP resting heat rate returned to its control value within 5–10 min. The effect of DNP on the rate of heat production of seven trabeculae is shown in Fig. 5 (□). The uncoupled heat rate per cm^3 tissue increased with decreasing radius (correlation coefficient −0·78). Since even the smallest preparations studied were well above the line calculated from Hill's equation it may be concluded that this dependency on radius was caused by anoxia at the core of the preparation.

DISCUSSION

Remarks on the technique

Our method to determine heat production in isolated cardiac muscle has several promising features. (i) The preparation is continuously superfused, which facilitates supply with substrate and removal of metabolites. (ii) The continuous measurement of heat rate is not disturbed by changing the superfusate. This means that the change in heat rate following a change of superfusate, i.e. the time course of the effect of intracellular concentration changes, can also be analysed. (iii) The signal-to-noise ratio is quite high, the background noise usually corresponding to less than 0·5 μW. There are three main noise sources that can interfere with the measurement: the temperature fluctuation of the chamber and the superfusate, the voltage noise of the differential amplifier recording the temperature signal, and variations in the rate of perfusion induced by the pump. At a perfusion rate of 1 μl/s the three noise sources produced voltage changes of the same order of magnitude, typically a few nanovolts peak-to-peak in the frequency range 0·01–1 Hz.

Metabolic rate of quiescent cardiac muscle

Table 1 summarizes values from the literature for energy output of resting myocardium between 27 and 37 °C from different mammalian species. To convert values from oxygen consumption to rates of heat production we used an energy equivalent of 20·3 J/ml O_2 (see e.g. Coulson, 1976). Most of the investigators quoted in Table 1 used glucose as sole substrate except Bünger, Permanetter, Sommer & Yaffe (1982) who used 5 mM-DL-3-hydroxybutyrate plus 1 mM-pyruvate and Rose & Kammermeier (1986) who used 5 mM-glucose plus 2 mM-pyruvate.

For easier comparison with the work of others we have converted our values for resting heat rate from mW/cm^3 to mW/g wet weight by dividing by 1·05. This gives a mean value of 22 mW/g with glucose as sole substrate. Since the working guinea-pig heart at normal pre- and after-load was found to expend between 59 and 69 mW/g (Loiselle & Gibbs, 1979; Bünger *et al.* 1982; Bardenheuer & Schrader, 1983; Bünger & Permanetter, 1984) the value of 22 mW/g is consistent with the notion that basal metabolism accounts for $\frac{1}{4}$ to $\frac{1}{3}$ of the metabolism of the beating heart (Gibbs, 1978). On the other hand our average value obtained with 20 mM-glucose plus 2 mM-pyruvate as substrates (34 mW/g) is, as far as we know, the highest metabolic rate of quiescent cardiac muscle measured so far. From the present experiments we cannot infer the precise reason why the values for resting metabolism of cardiac muscle vary so much and why our values are relatively high. However, it may be of interest to try to identify the experimental circumstances which could have enhanced the metabolic rate of the quiescent guinea-pig trabeculae in our study.

Factors influencing metabolic rate

Temperature. It is not very likely that the different temperatures at which the studies summarized in Table 1 were performed can explain much of the variability. Investigations by Loiselle (1985*a*, *b*) and by Loiselle & Gibbs (1983) show that the rates of resting oxygen consumption and heat production are relatively insensitive

to temperature. They found Q_{10} values of 1·3–1·4 in their experiments with rat and rabbit papillary muscle.

Diameter. We did not find a significant correlation between diameter and resting heat in the relatively small sample of preparations studied (Fig. 5). However, it has been shown previously that when a larger range of diameters is studied a statistically significant correlation between diameter and resting energy expenditure is obtained (Cranefield & Greenspan, 1960; Loiselle & Gibbs, 1983). We used muscles with an

TABLE 1. Metabolic rates of resting cardiac preparations

Species	Preparation	Temperature (°C)	Method	Heat rate (mW/g w.w.)	References
Rat	KCl-arrested whole heart	37	O_2 cons.	25·0	Penpargkul & Scheuer (1969)
Guinea-pig	KCl-arrested whole heart	37	O_2 cons.	22·0	Bünger *et al.* (1982)
Dog	KCl-arrested whole heart	37	O_2 cons.	5·9	Gibbs *et al.* (1980)
Rabbit	KCl-arrested whole heart	27	O_2 cons.	1·7	Gibbs & Kotsanas (1986)
Cat	Papillary muscle	37	O_2 cons.	3·8	Whalen (1960)
Cat	Papillary muscle	37	O_2 cons.	6·5	Lee (1960)
Cat	Papillary muscle	35	O_2 cons.	16·0	Cranefield & Greenspan (1960)
Kitten	Papillary muscle	35	O_2 cons.	22·8	Cranefield & Greenspan (1960)
Cat	Papillary muscle	27	Heat pr.	2·0	Loiselle & Gibbs (1979)
Rat	Papillary muscle	27	Heat pr.	6·2	Loiselle & Gibbs (1979)
Guinea-pig	Papillary muscle	27	Heat pr.	3·0	Loiselle & Gibbs (1979)
Rabbit	Papillary muscle	27	Heat pr.	2·6	Gibbs *et al.* (1984)
Rabbit	Papillary muscle	27	O_2 cons.	6·5	Gibbs *et al.* (1984)
Rat	Trabecula	37	O_2 cons.	8·3	Whalen (1960)
Rat	Myocytes	37	O_2 cons.	24·5	Montini *et al.* (1981)
Rat	Myocytes	37	O_2 cons.	7·3	Piper *et al* (1982)
Rat	Myocytes	37	O_2 cons.	11·5	Rose & Kammermeier (1986)
Rat	Myocytes	37	O_2 cons.	16·0	Kennedy & Jones (1986)

O_2 cons., O_2 consumption. Heat pr., heat production. w.w., wet weight.

average diameter of 364 μm, the thinnest preparations used so far in such studies. Thus it might be assumed that the small size of our preparations may have contributed to their high metabolic rate and to the lack of correlation between heat rate and diameter. Calculation of radial diffusion of oxygen in a cylindrical preparation (Hill, 1928, 1965) showed that only one of the fifteen trabeculae studied may have had an anoxic core (Fig. 5). In a recent series of experiments we have obtained evidence that the diffusion constant used is indeed applicable under our experimental conditions (Daut & Elzinga, 1987). The critical radius for a resting heat rate of 23 mW/cm^3 is 300 μm at 37 °C. Thus preparations with a radius larger than that would develop an anoxic core at that heat rate. However, even if the radius of the preparation was twice the critical radius the apparent heat rate per cm^3 would only be reduced by $\frac{1}{4}$ on account of the anoxic core. Thus it is unlikely that limited diffusion of oxygen in larger preparations can explain more than a small fraction of

the difference between our data and previous results. There remains, however, the possibility that larger preparations may have a lower metabolic rate due to reasons unrelated to an anoxic core.

Time after cardiectomy. We found no decay of heat production with time for up to 6 h after cardiectomy. In this respect our results are in agreement with the data of Loiselle & Gibbs (1979) who found that resting heat rate of guinea-pig papillary muscle did not change significantly between 1 and 4 h after cardiectomy. Although only three of our measurements started at 80 min after cardiectomy or earlier it seems likely that thereafter the resting heat rate of guinea-pig trabeculae is virtually constant. This is in agreement with measurements of oxygen uptake in superfused cat papillary muscle which was reported to be stable for several hours (Cranefield & Greenspan, 1960).

However, as far as the time dependence of resting heat production is concerned, guinea-pig cardiac preparations seem to be the exception amongst mammalian species rather than the rule. In thermopile experiments on isolated papillary muscles from rat, cat and rabbit a continuous decline of resting heat rate after the first measurement, which is usually taken 1 h after cardiectomy, has been found (Loiselle & Gibbs, 1979; Gibbs, 1983). Within 2–3 h after the first measurement resting heat rate declines to a more or less stable plateau of about 50% of the first measurement. It is the generally held view that a reliable estimate of basal metabolism can only be made after 3 h or so when its level has practically plateaued (Gibbs & Kotsanas, 1986). Such values are therefore reported in Table 1. However, since there is no good explanation for the decay of basal metabolism this seems to be a rather arbitrary choice. Thus the procedure chosen for evaluating the results represents another reason why our values appear to be inflated compared to data obtained with thermopiles for mammals other than guinea-pig.

Superfusion. Measurements on thermopiles are performed with the preparation out of solution. Gibbs *et al.* (1984) compared values for resting metabolism from thermopiles with those obtained from oxygen consumption measurements and found the latter to be about twice as high (see also Gibbs & Kotsanas, 1986). They related this finding to the fact that in the oxygen consumption experiments the preparations were continuously superfused. A possible reason for the apparent beneficial effect of continuous superfusion on isolated cardiac muscle could be that it facilitates the removal of cellular metabolites.

Substrate. Our finding that the rate of heat production at rest depends on the substrate composition of the superfusate (Fig. 6) corresponds in a general sense with a number of observations in the literature demonstrating substrate dependence of resting metabolism (Krebs, 1950; Chapman & Gibbs, 1974; Montini, Bagby & Spitzer, 1981; Gibbs & Kotsanas, 1986). The molecular basis of these large effects of substrate is still unclear; they may be partly due to differences in the ATP-to-O ratio.

From the considerations presented above it may be concluded that some, but not all, of the differences between our results and previous work can be explained by the temperature difference, the diameter, the procedure chosen to evaluate the results, continuous superfusion, and substrate composition. Across species differences will certainly also contribute to the variability of the results shown in Table 1. Another

possible complication is that there may be regional variation in the metabolism of the heart (see e.g. Rouleau, Paradis, Shenasa & Juneau, 1986).

Changing the superfusate

The heat production during replacement of external Na^+ by K^+ is probably mainly due to increased ATP turnover caused by normal cross-bridge cycling in the presence of a sustained increase in free intracellular calcium (Holubarsch, Alpert, Goulette & Mulieri, 1982; Sheu, Sharma & Uglesity, 1986). The time course of the heat produced during the high-K^+ contracture was found to be similar to the time course of isometric tension measured in sheep cardiac trabeculae (Gibbons & Fozzard, 1971) or cat papillary muscle (Morad & Rolett, 1972). The rate of heat production measured was larger by an order of magnitude than that measured during high-K^+ contractures of rat papillary muscle with thermopiles at 21 °C (Holubarsch *et al.* 1982). It should also be noted that our preparations were at slack length and that therefore the experiments reported here give no indication of the maximal heat rate the preparation can produce during activation of the actomyosin ATPase. The reason for the long-lasting change in resting heat rate found in some experiments after potassium contractures is not yet clear. Damage of the fibre due to a strong contracture against a very small load cannot be excluded. However, the fact that the increase in heat rate measured during the contracture remained rather constant despite the change in resting heat rate (see Fig. 7) would argue against such an interpretation.

Uncoupling of the mitochondria by application of 50 μM-2,4-dinitrophenol (DNP) increased the energy expenditure of the trabeculae considerably (values up to 170 mW/cm^3 were found). Even the thinnest trabeculae studied (250 μm diameter) must have developed an anoxic core under these conditions (see Fig. 5). This is the reason why the smaller trabeculae developed a larger heat rate per cm^3 of tissue during application of DNP. Isolated guinea-pig cardiomyocytes were found to consume 10 μl O_2 cm^{-3} s^{-1} during application of 50 μM-DNP (Spieckermann & Piper, 1985), which corresponds to 200 mW/cm^3. This agrees quite well with the estimate obtained by extrapolating the linear-regression line drawn through our data to the size of preparations not developing an anoxic core in the presence of DNP.

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