Thermochimica Acta, 193 (1991) 269–280
Elsevier Science Publishers B.V., Amsterdam

The contribution of the sodium pump to the basal metabolism of isolated cardiac muscle: a calorimetric study

Jürgen Daut [a], Tobias Groß [a] and Gijs Elzinga [b]

[a] *Physiologisches Institut der Technischen Universität München, Biedersteiner Str. 29, D-8000 München 40 (Germany)*
[b] *Laboratorium voor Fysiologie, Vrije Universiteit Amsterdam, Van der Boechorststr. 7, NL-1083 BT Amsterdam (Netherlands)*

(Received 25 February 1991)

Abstract

The rate of heat production of thin ventricular trabeculae isolated from guinea-pig heart was measured using a calorimetric technique. The preparations were mounted in a Perspex tube of 0.8 mm i.d. and 0.1 mm wall thickness. The tube was perfused with physiological salt solution at a constant rate of 1 μl s^{-1}. The temperature difference between two points on the Perspex tube, one upstream and one downstream of the preparation, was measured with constantan/chromel thermocouples, and was shown to be proportional to the rate of heat production of the preparation. Inhibition of the sodium pump in quiescent trabeculae with the cardiac glycoside dihydro-ouabain produced a decrease in the resting heat production rate of $4.1 \pm 1.0\%$. This probably represents the heat production related to the activity of the Na,K ATPase in the steady state, including the heat production due to the resynthesis of ATP via oxidative phosphorylation. From this figure, the turnover rate of the Na,K ATPase in resting cardiac muscle was calculated to be about 8 nmol cm^{-3} s^{-1}. Superfusion of beating preparations with dihydro-ouabain resulted in an approximately three-fold increase in the measured heat production rate. This effect was related to the positive inotropic action of the drug and may be a consequence of the increased activity of the actomyosin ATPase, the Ca ATPase, and the Na,K ATPase.

INTRODUCTION

It has long been known that contraction of isolated striated muscle is accompanied by chemical change and heat production [1]. Since then calorimetry has been widely used to elucidate the biochemical processes contributing to the conversion of chemical energy to mechanical work in various types of muscle [2–5]. In the classical calorimetric studies of muscle energetics, the total heat production was subdivided into functional entities such as the heat related to tension generation, activation heat, recovery heat, shortening heat, maintenance heat, etc. [2–4]. More recently, some groups have tried to subdivide the total heat production of muscle cells into

components attributable to individual enzymes [5,6]. If this could be achieved, a quantitative description of the metabolic processes in intact cells could be attempted with calorimetric methods, i.e. without disturbing the multiple feedback mechanisms in the cell and without disrupting the microenvironment of intracellular enzymes or the spatial inhomogeneities in the concentrations of various substrates and products.

In muscle, the chemical free energy derived from oxidation of substrates is converted to electrical, chemical and mechanical work. This energy transduction process is primarily mediated by the three major ATPases: the Na,K ATPase, the Ca ATPase and the actomyosin ATPase. In trying to correlate chemical changes with heat production, it is useful to consider the steady-state situation in an isolated piece of muscular tissue in a recording chamber superfused with oxygenated solution of constant pressure, temperature and composition. In such an open system the chemical composition of the cells may be assumed to be constant. When the recording chamber contains cells with a predominantly aerobic metabolism (such as cardiac muscle), the only work done by the system will be the oxidation of the metabolic substrate supplied with the solution (e.g. glucose) to CO_2 and H_2O, and all of the free energy change will be converted to heat. In the steady state, all of the ATP molecules hydrolysed per unit time are resynthesised by oxidative phosphorylation. Thus the heat production due to the action of an ATPase, including the heat production associated with the resynthesis of ATP, can be calculated from the equation

$$\Delta H_p = \Delta H_g / n \tag{1}$$

where ΔH_p(J mol^{-1}) represents the total molar heat production due to ATP hydrolysis and resynthesis, ΔH_g is the enthalpy of glucose oxidation and n is the number of molecules of ATP synthesised per molecule of glucose. At physiological concentrations of all intracellular reactants, n is generally assumed to be 38 and ΔH_g is about 2790 kJ mol^{-1}. Thus the heat production related to hydrolysis of ATP by intracellular ATPases in the steady state (including ATP resynthesis via oxidative phosphorylation) is 73 kJ per mol ATP. The rate of ATP synthesis is adapted to the rate of ATP hydrolysis by 'respiratory control'. The mechanisms responsible for respiratory control are not yet clear [7,8]. Nevertheless, as a first approximation, it may be assumed that small perturbations in the ATPase hydrolysis rate lead to corresponding changes in phosphorylation potential (and in phosphocreatine concentration) and, thus, in the rate of oxidative phosphorylation, and that a new steady state is reached within 30 s or so at 37 °C (see the Discussion section). In this idealised case, the changes in the rate of ATP hydrolysis might be inferred from the changes in heat production rate measured calorimetrically. An obvious candidate for testing this approach is the Na,K ATPase, because it can be selectively inhibited from the extracellular side of the cell membrane by application of cardiac glycosides.

With these considerations in mind, we have devised a calorimetric technique that allows continuous measurement of the rate of heat production of small superfused preparations of cardiac ventricular muscle [9,10]. A preliminary report of the present results has been published [11].

METHODS

Thin ventricular trabeculae less than 300 μm in diameter were isolated from guinea-pig heart as described previously [10]. A suitable preparation, twitching strongly upon electrical stimulation, was selected and mounted in a thin Perspex tube of 0.8 mm i.d. and 0.1 mm wall thickness. The tube was perfused with physiological salt solution (composition see below) at a constant rate by means of a suction pump (Precision pump M 16, Reichelt Chemie Technik, Heidelberg, Germany). 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 aluminum heated to 37°C (for details, see ref. 9). 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 illustrated in Fig. 1A. For simplicity, only two thermocouples on either side are shown. The thermocouples were embedded in the wall of the Perspex tubing and had no contact with the solution.

The heat produced by the preparation was taken up by the solution, which produced a temperature change according to the heat capacity of water. The temperature of the thin Perspex wall of the tubing followed the temperature of the solution virtually instantaneously, which caused the downstream thermocouples to become a little warmer than the upstream thermocouples. The relation between the rate of heat production of the preparation $\dot{H}$ (W) and the measured temperature change can be described by

$$\dot{H} = \Delta T f C_h / y \tag{2}$$

where ΔT is the temperature difference between the upstream and the downstream thermocouples (K), f is the flow rate of the perfusing solution ($cm^3\ s^{-1}$), C_h is the heat capacity of water ($J\ K^{-1}\ cm^{-3}$) and y is the yield of the system (dimensionless), i.e. the fraction of the applied heat that is actually recorded by the thermocouples. The system was calibrated by introducing a small thermistor bead into the recording chamber and applying a known heat production rate by means of a constant voltage source. The yield of the system was 0.77 for a heat source positioned exactly between the two sets of thermocouples, i.e. the measured temperature difference was 77% of that expected for a system with zero heat loss (see refs. 9 and 10).

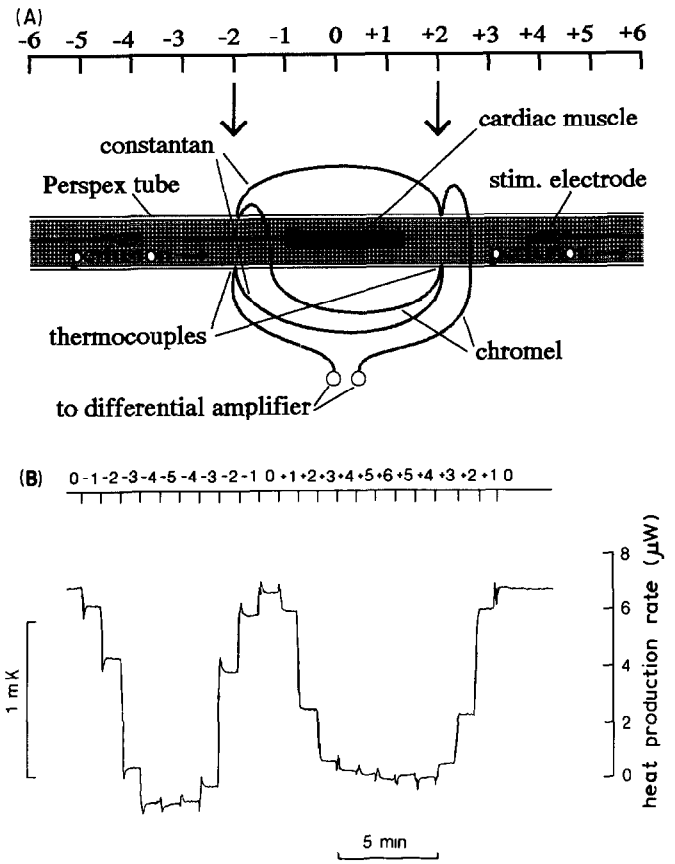

Fig. 1. (A) Schematic diagram of the recording chamber (see text). The coordinate on top indicates the position of the trabecula, i.e. the distance of its midpoint from the centre of the chamber (in mm). The position exactly between the two sets of thermocouples was defined as position 0. There were six chromel/constantan thermocouples spaced regularly at angles of 60° around the circumference of the tube. For simplicity, only two are shown on either side (vertical arrows). (B) Measurement of the rate of heat production of a trabecula as a function of its position in the recording chamber. At intervals of 1 minute the trabecula was shifted first in the upstream direction (left, negative numbers) and subsequently in the downstream direction (right, positive numbers) and back to position 0.

In order to obtain an absolute value of the power output of the preparation, a point of reference is required, i.e. a potential difference between the two measuring points (see Fig. 1) which corresponds to zero heat production of the preparation. This was obtained by moving the preparation along the longitudinal axis of the recording chamber with two manually operated microdrives (Mitutoyo). The ends of the preparation were connected to two platinum wires (50 μm diameter) by means of two fine Nylon threads (Fig. 1A). The Pt wires, which also served as stimulating electrodes, were attached to the two microdrives (Fig. 1A) and were positioned outside the inner part of the recording chamber in order to reduce the heat artefacts caused by

current flow across the resistance of the Pt/saline interface. With these precautions, electrical stimulation of the preparation (duration, 0.5–1.0 ms; amplitude, 3–4 V) produced no detectable heat artefact in the recording system [10].

The position of the midpoint of the preparation was defined by the coordinates shown in Fig. 1A above the recording chamber. Position zero is the midpoint of the chamber, i.e. at position zero the preparation is exactly at the mid-point between the two sets of thermocouples. Position +1 is 1 mm in the downstream direction, position −1 is 1 mm in the upstream direction, etc. The potential difference recorded when the preparation was pulled out of the recording chamber in the downstream direction (position +5) represents the baseline, i.e. zero power output. This is indicated by the ordinate on the right-hand side of Fig. 1B.

When the preparation was pulled out on the upstream side of the recording chamber (position −5), the measured potential difference was more negative than at position +5. In the ideal case with zero heat loss across the wall of the chamber, the thermocouples should record the same temperature difference with the preparation at positions +5 or −5. In reality, the potential difference was shifted in the negative direction at position −5, i.e. the preparation caused the upstream thermocouples to become a little warmer than the downstream thermocouples. If we take the potential recorded at position +5 as zero, the ratio between the signal recorded at position 0 and at position −5 was −0.2/1.0. This indicates that about 20% of the temperature increase caused by the preparation at position −5 was lost across the wall of the Perspex tube between the two sets of thermocouples. From these observations the thermal length constant of the system can be estimated to be at least 100 mm, which is consistent with the high yield of the system [9,10]. These considerations confirm the idea that only a small fraction of the heat produced by the preparation is lost across the wall of the tubing. At the velocity of the solution chosen (2 mm min^{-1}), the radial diffusion of the heat in the perfusing solution and the charging up of the heat capacity of the wall of the chamber are fast enough to give a response that is 90% complete in 1.5 seconds [10]. The rate of perfusion and the dimensions of the tubing were chosen in such a way that the longitudinal diffusion of heat in the wall of the tubing was negligible compared to the heat exchange in the radial direction. This is the reason why the temperature change of the wall of the tubing was found to be proportional to the rate of heat production of the preparation.

The size of the preparations from which meaningful signals can be recorded is limited by the radial diffusion of oxygen into the preparation (for details, see ref. 10). The preparations used in the present study weighed between 200 and 600 μg. For studying the metabolic activity of such small preparations, a high sensitivity of the recording system was required. Changes in heat production rate of less than 100 nW could be recorded at a

bandwidth of 0–10 Hz. This corresponds to temperature changes of less than 20 μK. The two main noise sources remaining after careful thermal isolation of the system were the voltage noise of the amplifier and the slight variation of the flow rate produced by the suction pump [9].

The perfusing solution had the following composition (mM): 116 NaCl, 4 KCl, 2 $CaCl_2$, 0.8 $MgCl_2$, 1 NaH_2PO_4, 24 $NaHCO_3$ and 10 glucose. The solutions were pre-heated to 37 °C and equilibrated with 95% O_2 and 5% CO_2; the pH was 7.4. The calorimeter was continuously perfused with two different solutions, one flowing through the recording chamber and the other going to waste. Both channels were connected to a suction pump driven at a speed of 1 μl s^{-1}. The solution could be changed with a four-way stopcock located 2 cm before the Perspex tube in which the preparation was mounted. Switching between different solutions did not interfere with the measurements [9]. The cardiac glycoside dihydro-ouabain (DHO), which was used to block the sodium pump in the cell membrane of the myocardial cells, was a gift from Hommel AG, CH-8134 Adliswil, Switzerland. The experiments were carried out partly at the Free University in Amsterdam and partly at the Physiological Institute of the Technical University in Munich, with two very similar calorimeters.

RESULTS

In every experiment the resting heat production rate was determined by moving the quiescent (i.e. not stimulated) preparation from position 0 to position +5 and back again, as illustrated on the right-hand side of Fig. 1B. This determination of the baseline was repeated every hour in order to detect any drift or any small changes in resting heat production rate. The resting heat production rate, which represents the basal metabolism of the cardiac cells, did not change significantly during experiments lasting up to 10 hours [9]. When the preparation was stimulated electrically the measured heat production rate increased. Figure 2A shows an experiment in which a preparation was paced at a rate of 3 Hz. The electrical stimulation was switched off at the beginning of the recording (downward arrow) and switched on again at the end of the recording (upward arrow). The rate of heat production in the beating preparation was about 5 μW and the resting heat production rate was about 3 μW. The relative increase in heat production depended on the rate of stimulation [10].

The contraction-related heat production is mainly attributable to the increased energy expenditure of three enzymes: the actomyosin ATPase, which reflects the increased activity of the contractile proteins; the Ca ATPase, which mediates the cycling of calcium between cytoplasm and sarcoplasmic reticulum; and the Na,K ATPase, which is required to pump out the sodium ions entering the cells during the action potential. We tried to inhibit the sodium pump completely by superfusing the preparations with

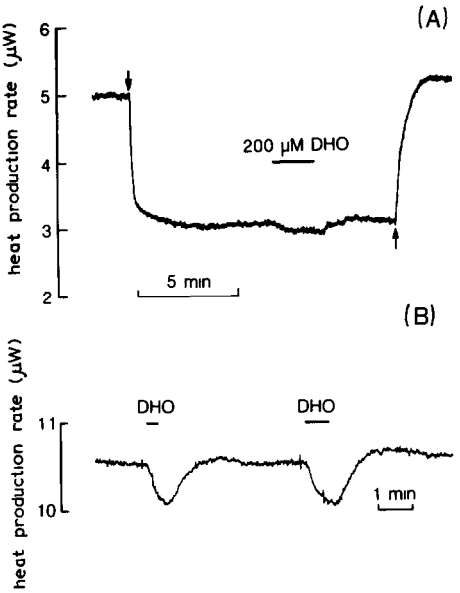

Fig. 2. The effects of dihydro-ouabain (DHO) on the resting heat production rate of isolated cardiac muscle. (A) Application of 200 μM DHO in the interval between two periods of stimulation (3 Hz, left and right). (B) Application of 200 μM DHO in a different quiescent trabecula. In this experiment the potassium concentration of the perfusate was reduced from 4 to 0.6 mM during application of DHO.

200 μM dihydro-ouabain (DHO), a fast-acting cardiac glycoside. Electrophysiological experiments have shown that the Na,K pump of thin ventricular trabeculae is blocked almost completely in the first 15–30 seconds after application of 200 μM DHO [12]. It can be seen in Fig. 2A that during application of DHO there was a small but distinct and reversible decrease in heat production rate. This was a consistent finding. Great care was taken to eliminate any switching artefacts [9]. The result was exactly the same when the two channels containing the solutions with and without DHO were interchanged. The recordings were filtered at 1 Hz.

In order to make sure that the Na,K pump was blocked completely, we reduced the potassium concentration of the perfusate during application of DHO from 4 to 0.6 mM in three experiments. The reduction of extracellular potassium greatly increases the affinity of the binding of DHO to the Na,K ATPase [13]. The reduction in heat production rate measured with 0.6 mM K^+ + 200 μM DHO (Fig. 2B) was very similar to that obtained with application of DHO at constant extracellular potassium (Fig. 2A). Reduction of external K^+ from 4 to 0.6 mM alone had no measurable effect on the resting heat production rate. In eight preparations, the decrease in resting heat production rate in the time period 1–2 min after application of 200 μM DHO was determined; on average, it decreased by 4.1 $\pm$ 1.0% (mean $\pm$ SD) after application of DHO.

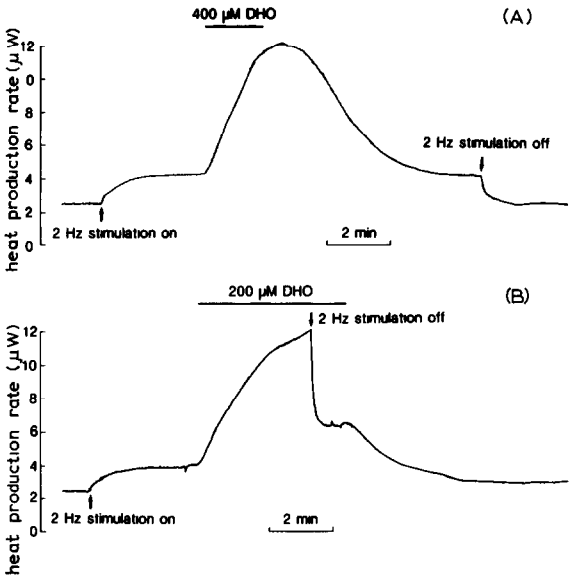

Fig. 3. Continuous recording of the rate of heat production of an isolated cardiac ventricular trabecula. At the times indicated by the vertical arrows the stimulation was switched on or off. During the period indicated by the horizontal bars the perfusate contained 400 μM (A) or 200 μM (B) DHO.

When the cardiac glycoside was applied during electrical stimulation of the preparation, the results were quite different. Figure 3A shows that the rate of heat production increased about three-fold within 2 minutes after application of 400 μM DHO. As the maximal heat production rate recorded is limited by the radial diffusion of oxygen into the preparation [10], the measured change in heat production rate represents a lower limit of the increase in energy expenditure that can be induced in contracting cardiac muscle cells by blockage of the sodium pump. After removal of the drug, the heat production rate decayed to its previous steady-state value within 10 minutes. After switching off the electrical stimulation, the measured heat production rate returned towards its basal value within 2 minutes.

It is generally agreed that the positive inotropic effect of cardiotonic steroids is related to the inhibition of the Na,K pump. The inhibition of Na extrusion across the cell membrane causes intracellular Na^+ accumulation, which leads to increased net Ca^{2+} influx via the Na,Ca exchanger. The resulting increased intracellular Ca^{2+} cycling between sarcoplasmic reticulum and cytoplasm is thought to be responsible for the positive inotropic effect. In order to characterise the effects of intracellular ionic changes on the heat production of a quiescent preparation, we modified slightly the experiment shown in Fig. 3A. About 10 minutes after the recording shown in Fig. 3A, dihydro-ouabain was applied again, as illustrated in Fig. 3B. This time only 200 μM DHO was used, which resulted in a somewhat slower rise

in heat production rate. When the heat production rate had reached 12 μW, the stimulation was switched off in the continued presence of DHO. It can be seen that the heat production rate decayed to a new steady-state value of about 6 μW. After removal of DHO, the rate of heat production returned to its basal value. This result suggests that the increased heat production rate observed after switching off the electrical stimulation related to the intracellular accumulation of Na^+ and Ca^{2+}. The rise in intracellular Ca^{2+} may have increased the energy expenditure of the cells by stimulating the Ca ATPase (see the Discussion section).

DISCUSSION

The calorimetric method described here has some advantages over the traditional method for analysing energy expenditure of muscle by means of thermopiles (reviewed in ref. 2–4). The main difference between Hill's traditional method and our new approach is that the temperature changes of the thermopiles represent heat (analogous to charge) whereas the temperature changes measured in our system represent heat production rate (analogous to current). The reason for this is that the heat capacity of the thermopiles is fixed (although it has a leak, which complicates the quantitative analysis) whereas in our method the heat capacitor (the solution) is flowing past the preparation at a constant velocity. A continuous row of infinitely small capacitors making brief contact with a current source is equivalent to a resistor, hence the temperature change measured (analogous to voltage change) is proportional to the heat carried away by the solution per unit time (analogous to current), and not to heat (analogous to charge). Thus our method gives a continuous measurement of the rate of heat production of small pieces of living tissue.

A second advantage of our approach is that the preparations of isolated cardiac muscle are continuously superfused, which prevents substrate depletion and allows switching between different solutions without interfering with the measurements. The sensitivity of the system is high enough to allow reliable determination of the resting heat production rate of myocardial preparations weighing less than 50 μg. The frequency resolution of our system, however, is lower than that of small thermopiles with a very low heat capacitance. In our calorimeter, step changes in heat production rate give voltage changes that reach 90% of their steady-state value within 1.5 s [10]. This is sufficient to detect the heat production of single twitches of small cardiac ventricular preparations at 37 °C.

We have measured the change in basal metabolism induced by inhibition of the sodium pump with dihydro-ouabain (DHO). One minute after application of DHO, the measured heat production rate had decreased by $4.1 \pm 1.0\%$. The average value of resting heat production rate with 10 mM glucose as substrate was about 14 mW cm^{-3} of tissue [10]. Thus the decrease

in the rate of heat production of quiescent cardiac produced by DHO, $\dot{H}_{DHO}$, was about 0.6 mW cm^{-3}. Our data may be compared with results obtained on mouse soleus muscle at 30 °C [14]. In this study (using a different microcalorimetric technique), the resting heat production rate was found to be decreased by 0.22 mW (g wet weight)$^{-1}$ during inhibition of the sodium pump, which amounts to about 9% of the resting heat production rate.

As noted in the Introduction (eqn. (1)), the decrease in heat production rate observed in the first two minutes after application of DHO probably represents the total effect of inhibition of the sodium pump and the subsequent change in ATP synthesis. The validity of eqn. (1) depends critically on the assumption that the molar heat production associated with the hydrolysis of ATP, ΔH_p (J mol^{-1}), is the same under control conditions in quiescent myocardial preparations and during our measurements of the effects of DHO. This does not imply that the phosphorylation potential (and the creatine/phosphocreatine ratio) have to remain constant after inhibition of the Na,K pump. However, it is necessary to assume that these changes have reached a steady state within 1 minute, which implies that the delay between inhibition of the sodium pump and the subsequent changes in oxidative phosphorylation is well below 1 minute. The fact that the change in electrogenic pump current recorded under very similar experimental conditions [12] had a time course very similar to the changes in heat production rate described here is consistent with this assumption. Another necessary condition for the validity of eqn. (1) is that the possible changes in intracellular Ca^{2+} evoked indirectly by inhibition of the Na,K pump do not have any major effect on resting heat production rate in the first two minutes after application of DHO. At present there are no direct measurements available to verify or contradict this.

If the assumptions outlined above are correct, the amount of ATP hydrolysed per second by the sodium pump in resting cardiac muscle cells, N_{ATP} (mol s^{-1} cm^{-3}) can be calculated from the decrease in the specific heat production rate observed during the application of DHO, $\dot{H}_{DHO}$ (W cm^{-3}), using the equation [6,15]

$$N_{ATP} = \dot{H}_{DHO}/\Delta H_p \tag{3}$$

where ΔH_p is defined by eqn. (1). The rate of ATP hydrolysis by the sodium pump calculated from eqn. (3) was 8 nmol cm^{-3} s^{-1}.

The interpretation of our experiments on beating preparations is more difficult because the relative contributions of the Ca ATPase and the actomyosin ATPase to the total heat production are not yet known. Preparations of up to 300 μm diameter stimulated at a frequency of 2–3 Hz are adequately supplied with oxygen by diffusion from the perfusate [10]. Thus it may be assumed that their heat production rate in the steady state was approximately proportional to the rate of ATP hydrolysis. During the

marked increase in energy expenditure induced by high concentrations of cardiac glycosides (Fig. 2), the larger preparations (> 200 μm diameter) probably developed an anoxic core. This induces radial gradients in pH, ion concentrations and phosphorylation potential, which makes a quantitative analysis impossible.

Comparison of Figs. 3A and 3B provides some interesting inferences on the effects of intracellular Na and Ca ions on basal metabolism. When the stimulation was interrupted in the continued presence of DHO, the measured heat production rate reached a plateau that was more than twice as high as the resting heat production rate measured under control conditions. The increase in intracellular Na^+ caused by prolonged blockage of the sodium pump is expected to induce an increase in free intracellular Ca^{2+} mediated by trans-membrane Na,Ca exchange. The observation that the activity of the sodium pump was necessary to reverse the increase in heat production rate suggests that the driving force for Ca^{2+} extrusion is to a large extent provided by the Na,K pump. The increase in the trans-membrane Na^+ gradient resulting from the extrusion of sodium ions is expected to induce a Ca^{2+} efflux via the Na,Ca exchanger. These considerations suggest that the elevation of heat production rate after a period of increased activity is at least partly mediated by the concomitant rise in free intracellular Na^+ and Ca^{2+}, which activates the Na,K ATPase and the Ca ATPase. Further experiments are required to quantify the contribution of these enzymes to the energy expenditure of the beating heart.

The results presented above show that our calorimetric method may be suitable for determining the turnover rate of the Na,K ATPase in quiescent cardiac muscle. In principle, the technique can also be applied to other enzymes, and to all types of cells. A particularly interesting possibility is to introduce clones of cultured cells attached to microcarrier beads into the recording chamber. So far only membrane-bound proteins, e.g. ion channels, have been amenable to quantitative analysis of the results of genetic modifications. With the improvement of calorimetric techniques, alterations in the turnover rate of intracellular enzymes in intact cells could also be studied.

ACKNOWLEDGEMENTS

We than Ronald Buitenweg and Ludwig Müller for building the two calorimeters. Without their skill and expertise this work would have been impossible. We also thank Prof. J. Dudel for reading the manuscript. The work was supported by the Deutsche Forschungsgemeinschaft (Da 177/2-4).

REFERENCES

1 H. Helmholtz, Über die Wärmeentwicklung der Muskelaction, Müller's Archiv für Anatomie und Physiologie, (1848) 144–164.

2 A.V. Hill, Trails and Trials in Physiology, Arnold, London, 1965.
3 C.L. Gibbs, Physiol. Rev. 58 (1978) 174–254.
4 R.C. Woledge, N.A. Curtin and E. Homsher, Energetic Aspects of Muscle Contraction, Monographs of the Physiological Society No. 41, Academic Press, London, 1985.
5 C.L. Gibbs and J.B. Chapman, Cardiac Energetics, in Handbook of Physiology, Cardiovascular System, section 2, Vol. 1. American Physiological Society, Bethesda, 1979, pp. 775–804.
6 J. Daut, The energetics of the Na,K-pump in cardiac muscle, in H.Ch. Lüttgau (Ed.), Fortschritte der Zoologie, Vol. 33, Membrane Control of Cellular Activity, Fischer Verlag, Stuttgart, 1986, pp. 419–427.
7 I. Hassinen, Mitochondrial respiratory control in the myocardium, Biochim. Biophys. Acta, 853 (1986) 135–151.
8 F.W. Heineman and R.S. Balaban, Control of mitochondrial respiration in the heart in vivo, Annu. Rev. Physiol. 52 (1990) 523–542.
9 J. Daut and G. Elzinga, Heat production of quiescent ventricular trabeculae isolated from guinea pig heart, J. Physiol., 398 (1988) 259–275.
10 J. Daut and G. Elzinga, Substrate dependence of energy metabolism in isolated guinea-pig cardiac muscle: a microcalorimetric study, J. Physiol., 413 (1989) 379–397.
11 J. Daut and G. Elzinga, Heat production of the sodium pump in guinea-pig cardiac muscle, Pflügers Arch., 411 (1988) Suppl. 1, R12.
12 J. Daut and R. Rüdel, The electrogenic sodium pump in guinea-pig ventricular muscle: inhibition of pump current by cardiac glycosides, J. Physiol., 330 (1982) 243–264.
13 J. Daut, Inhibition of the sodium pump in guinea-pig ventricular muscle by dihydroouabain: effects of external potassium and sodium, J. Physiol., 339 (1983) 643–662.
14 A. Chinet, T. Clausen and L. Girardier, Microcalorimetric determination of energy expenditure due to active sodium–potassium transport in the soleus muscle and brown adipose tissue of the rat, J. Physiol., 265 (1977) 43–61.
15 J. Daut, The living cell as an energy-transducing machine. A minimal model of myocardial metabolism, Biochim. Biophys. Acta, 895 (1987) 41–62.