Journal of Supramolecular Structure 3:175–180 (1975)

HEAT PRODUCTION AND METABOLISM DURING THE CONTRACTION OF MAMMALIAN SKELETAL MUSCLE

K. M. Kretzschmar

Department of Physiology, University College, London

Methods are described whereby initial processes of muscular contraction may be investigated in a mammalian preparation, the soleus muscle of the rat. Conditions are chosen so that recovery is avoided. An isometric tetanus is investigated and an energy balance sheet is drawn up. It is found that there is more heat evolved than can be accounted for in terms of measured chemical reaction. This discrepancy is discussed with reference to the similar results that have been obtained using frog muscle.

INTRODUCTION

During the past few years it has become increasingly clear that there are discrepancies between in vivo measurements of the chemical and thermal changes accompanying muscular contraction and in vitro measurement of the relevant enthalpy changes. Such discrepancies may be divided into two broad classes: (a) those found in short or incomplete contractions (see for example, references 1–3); (b) those found in longer contraction and relaxation cycles. The present paper is concerned primarily with the latter class, in which the discrepancies are large, and theoretical interpretation is simplified because there is presumably no net change in the condition of the contractile proteins or in the distribution of calcium ions within the cell.

In vivo measurements, under conditions where hydrolysis of phosphocreatine (PCr) is thought to be the only net reaction, yield a ratio of heat + work production (h + w) to phosphocreatine breakdown (ΔPCr) of about −46 kJ/mol (4). In contrast, in vitro determinations of the enthalpy change that is associated with PCr hydrolysis under in vivo conditions suggest a value of −34 kJ/mol (Woledge references 5–6, and personal communication). Thus, there is more energy produced during a muscular contraction than can be accounted for by known chemical reactions. In this paper I shall present some previously unpublished results that shed further light on this problem, and discuss the conclusions that may be drawn from the relevant data available at present.

METHODS AND RESULTS

A mammalian muscle has been used in an investigation of energy balance during muscular contraction. Heat (for methods see reference 7) and chemical measurements

Present address: Cardiovascular Research Institute, University of California, San Francisco, California

 © 1975 Alan R. Liss, Inc., 150 Fifth Avenue, New York, N.Y. 10011

were made 3 sec after a 10 sec isometric tetanus at 17–18°C in nitrogen using rat soleus muscles at optimum muscle length for tension development (about 27 mm for a muscle weight of 86 mg). Under these conditions the muscles contract normally: the peak isometric tension (P) is satisfactory (Pl/m = 23.2 ± 1.1 N cm^{-2}, mean and SE, N = 6. l = length of muscle, m = muscle weight); rise of tension and relaxation are simply slowed by the low temperature used. Oxidative recovery is absent and glycolysis is so slow that it accounts for only a small fraction of the energy output. Anoxia does not effect the resting level of phosphocreatine (PCr/Ct = 0.681 ± 0.013, mean and SE, N = 17. PCr = phosphocreatine, Ct = total creatine = PCr + free creatine).

After arrest of metabolism using the hammer apparatus (8), protein-free muscle extracts were prepared as described by Dydynska and Wilkie (9) and were subsequently analyzed for PCr and Ct (9, 10), and for ATP, adenosine diphosphate, adenosine monophosphate, glucose-6-phosphate, fructose-6-phosphate, fructose-1, 6-diphosphate, lactate, phosphoenolpyruvate, and pyruvate (11). Total adenine and inosine were measured spectrophotometrically using a modification (Woledge, personal communication) of the method of Kushmerik and Davies (12). The treatment of the muscle with nitrogen produced no sizable change in the level of any metabolite. The metabolic consequences of contraction are shown in Table I. Only four metabolites are tabulated; analyses for the other compounds listed above gave mean changes that were very small and not significantly different from zero. The heat production expected as a result of each reaction is calculated and given in Table I. The sum of these, the total heat expected from measured reactions, is clearly less than the heat production that was observed (see Table II) (there is no net work done, so all energy output is in the form of heat). The difference amounts to almost 2000 mJ/mmol Ct, about 30% of the heat evolved, and is significantly different from zero ($P < 0.01$).

Another way of expressing the results, that allows comparison with previous results, is to calculate the heat production expected from all reactions but PCr splitting and to subtract this figure from the measured heat. The result (6,309 ± 423 mJ/mmol Ct, mean and SE) is that portion of the heat production that it is reasonable to compare with ΔPCr. The ratio thus obtained is –49.7 ± 5.58 (mean and SE) kJ of heat production per mol of PCr breakdown, which is significantly different from –34 kJ/mol (the enthalpy change for PCr hydrolysis) but is not significantly different from –46 kJ/mol (the value for this ratio obtained in frog muscle by Wilkie (4). Thus, it seems that experiments performed on muscles from species as different as frog and rat yield the same energy output to ΔPCr ratio, but that this ratio does not agree with the in vivo enthalpy change associated with PCr hydrolysis.

DISCUSSION

The existence of a discrepancy between energy output and chemical change in complete contraction and relaxation cycles seems to be the major puzzle in the field of muscle energetics at present. Therefore its origin is discussed in some detail below.

Experimental Error

The conclusion that there is a lack of energy balance depends on measurement of the absolute values of heat production, chemical change, and the relevant in vivo enthalpy

TABLE I. Chemical Change During a 10 Sec Isometric Tetanus

			Phosphocreatine	Lactate	Glucose-6-phosphate	Fructose-1, 6-diphosphate
Chemical change	μmol/mmol Ct	Mean	−126.89	+ 2.39	+ 0.87	+ 1.50
		SE	11.42	1.16	0.46	0.54
		N	10	11	12	12
		P		0.1–0.05	0.1–0.05	< 0.02
ΔH	kJ/mol		− 34	−96	−12.5	~ 0
Expected heat	mJ/mmol Ct	Mean	4315	229	11	0
		SE	388	111	5	0

All measurements of chemical change are normalized by the total creatine content (Ct = free creatine + phosphocreatine) of muscle. The changes may be expressed in μmol/gm, using the Ct content of these muscles (16.76 ± 1.03 μmol/gm wet weight of muscle, mean and SE, N = 6) but this introduces additional random error. A negative sign indicates breakdown, a positive indicates synthesis. Values for the enthalpy changes (Δ H) are those given by Woledge (1971, 1972, personal communication). The expected heats are the products of the chemical change and the appropriate Δ H. See text for experimental details.

TABLE II. Expected and Observed Heat Productions During a 10 Sec Isometric Tetanus

	mJ/mmol Ct		
	Heat expected	Heat observed	Difference
Mean	4,555	6,549	1,994
SE	404	408	574

All quantities are normalized by the total creatine content (Ct = free creatine + phosphocreatine). The expected heat is the sum of the expected heats shown in Table I. See text for experimental details.

changes. It could be that the discrepancy is an artifact caused by inaccurate measurement. A calibration error of about 30% would be required to explain away the discrepancy.

Measurement of heat. There is little doubt that the Seebeck coefficient for the type of thermopile used in our laboratory is known accurately. Calibrations by methods of immersing in baths at different temperatures (13), and by a method using the Peltier effect in the thermojunctions themselves (7) agree accurately with the tabulated thermal parameters of the materials used, namely chromel and constantan (13). Thus, temperature may be measured accurately. To convert such a measurement to one of heat, the thermal capacity of the muscle/thermopile assembly is required. This quantity may be found by calculation from the thermal parameters of the constitutents of the assembly (13) or by liberating a known quantity of heat in a resistance wire situated in the thermopile (14) or by passing a current through the thermopile itself, and liberating or absorbing a known quantity of heat at the thermojunctions by virtue of the Peltier effect (7). These three methods give very similar results when used in conjunction with frog sartorius muscle. The heat calibration employed in the experiment on the rat soleus described above used the Peltier effect. As a further check on the accuracy of calibration, a number of

workers in our laboratory have used "dummy muscles." These consist of resistance wires suitably embedded and mounted on the thermopile undergoing calibration. The dummy produces a known quantity of heat which is measured independently by the thermopile. The two figures thus obtained agree within experimental error, and certainly there is no suggestion of an error large enough to explain the observed discrepancy.

Measurement of metabolism. In many experiments the sole or major chemical change was hydrolysis of PCr (see references 1 and 4, and the work reported above); in these experiments the discrepancy cannot be explained in terms of a calibration error in the measurement of compounds other than PCr. The addition of known quantities of PCr or creatine to muscle extracts has shown the analysis for PCr to be reliable and accurate. The sensitivity of the method has remained practically constant over a number of years. Likewise, the recovery of PCr or creatine, added to muscle before the preparation of protein free extracts, is satisfactory and does not indicate the disappearance of PCr (e.g., via hydrolysis) during extraction. We have no way of knowing whether PCr is broken down during freezing before extraction. However, some batches of sartorius muscles from the frog show a ratio of PCr to free creatine as high as 9:1, so at most 10% of the PCr could have been hydrolyzed. In contrast the discrepancy could only be explained in terms of a 30% calibration error. Indeed, since all our results are expressed as differences between paired muscles, a fixed amount of PCr breakdown during freezing would produce no error in the results.

The Enthalpy Change Associated with PCr Hydrolysis In Vivo

The accuracy of the value derived from measurements in vitro and used for the enthalpy change associated with PCr splitting in vivo (ΔH_{obs}) has been discussed in detail by Woledge (5, 6), and it is unnecessary to repeat those arguments here. Suffice it to say that it is difficult to imagine how a large error could arise. Certainly the value obtained for ΔH_{obs} is not particularly sensitive to errors in parameters such as pH and pMg, and even extreme assumptions cannot yield figures as large as –46 kJ/mol.

The values for ΔH_{obs} at both 0°C and 25°C have been computed. It is interesting, therefore, that energy balance experiments have been performed on frog muscle at 0°C (4) and at 20°C (15), and on rat muscle at 17–18°C. At each temperature a similar discrepancy is apparent.

The Source of the Discrepancy

If one accepts that there is no experimental error involved in the drawing up of the energy balance sheet, then one is driven to the conclusion that an unidentified source of energy manifests itself during muscular contraction. The nature of this source of energy is discussed below.

Activation. The evidence currently available, although sketchy, suggests that the energetics of activation holds no surprises. Gibbs and Seraydarian (16) report that the binding of calcium to the sarcoplasmic reticulum produces only little heat and that the value obtained for the enthalpy change due to ATP hydrolysis during calcium pumping in isolated sarcoplasmic reticulum agrees closely with the in vitro value for the same quantity, measured by Podolsky and Morales (17). However, let us make the opposing hypothesis, namely that the observed discrepancy is associated solely with activation.

Homsher et al. (18) find that approximately 30% of the heat produced during an isometric tetanus is associated with activation. The remaining 70% of the heat is associated with the mechanical response which, we have postulated, involves no unknown source of energy. Thus, the observed PCr breakdown is just sufficient to account for crossbridge activity (at −34 kJ/mol) and there remains no PCr hydrolysis to allow for the ATP splitting that accompanies calcium pumping (19). Therefore, the hypothesis is untenable and (if activation heat is 30% or less of the total heat production) at least a portion of the energy unaccounted for must arise from processes other than activation.

The Lohmann reaction (PCr + Adenosine diphosphate ⇌ ATP + creatine). It might be that the discrepancy is associated with PCr hydrolysis, but not with ATP splitting itself. PCr appears to be bound to muscle proteins (20); perhaps it unbinds with appropriate heat liberation upon hydrolysis. The evidence is not conclusive: Homsher et al. (18) find no discrepancy in the energy balance of muscles in which the Lohmann reaction is prevented by the use of FDNB. However, this result was obtained by using muscles stretched so that actomyosin interaction could not occur, and so the interpretation is not certain. Curtin and Woledge (in press) have investigated contraction in the presence of FDNB at normal muscle lengths, and find a discrepancy at least qualitatively similar to that observed under conditions where PCr is hydrolyzed.

Other sources of energy. During discussion following presentation of this paper, points were raised that are worthy of mention here.

It was suggested that the source of the excess energy might be an enthalpy change associated with the acylation of glyceraldehyde-3-phosphate dehydrogenase, a process that might precede any detectable glycolysis (see, for example, reference 21). This explanation may be excluded, since muscles treated with iodoacetate so that glyceraldehyde-3-phosphate dehydrogenase is inhibited nonetheless exhibit the usual unexplained energy production.

It was suggested that the potential across cell membranes was a form of energy not considered in the energy balance, and a change in this potential might give rise to the unexplained energy. However, such a possibility is out of the question: the energy stored in the resting potential represents less than 0.1% of the energy to be accounted for. Activity of the sodium-potassium exchange pump would result in ATP breakdown, and therefore would have been taken into account in drawing up the energy balance.

CONCLUSIONS

There is strong evidence for the existence of an unidentified process that provides a sizable fraction of the total energy output during muscular contraction. It seems that processes ancillary to tension transduction, such as electrical activity, calcium movements and recovery are unlikely sources for the unexplained heat. It remains possible that these experiments disclose the presence of an unknown process that is associated with the contractile event itself.

ACKNOWLEDGMENTS

I would like to thank David Gower for his help with the chemical analyses and the preparation of muscle extracts.

REFERENCES

1. Gilbert, C., Kretzschmar, K. M., Wilkie, D. R., and Woledge, R. C., J. Physiol. 218:163 (1971).
2. Curtin, N. A., Gilbert, C., Kretzschmar, K. M., and Wilkie, D. R., J. Physiol. 238:455–472 (1974).
3. Homsher, E., Rall, J. A., Wallner, A., and Ricchiuti, N. V., J. Gen. Physiol. 65:1–21 (1975).
4. Wilkie, D. R., J. Physiol. 195:157–183 (1968).
5. Woledge, R. C., Prog. Biophys. Mol. Biol. 22:37–74 (1971).
6. Woledge, R. C., Cold Spring Harbor Symp. Quant. Biol. 37:629–634 (1972).
7. Kretzschmar, K. M., and Wilkie, D. R., J. Physiol. 224:18P (1972).
8. Kretzschmar, K. M., and Wilkie, D. R., J. Physiol. 202:66P (1969).
9. Dydynska, M., and Wilkie, D. R., J. Physiol. 184:751 (1966).
10. Ennor, A. H., and Rosenberg, H., Biochem. J. 51:606–610 (1952).
11. Scopes, R. K., Anal. Biochem. 49:73–87 (1972).
12. Kushmerick, M. J., and Davies, R. E., Proc. R. Soc. Lond. Ser. B. Biol. Sci. 174:315–353 (1969).
13. Hill, A. V., "Trails and Trials in Physiology," Arnold, London.
14. Wilkie, D. R., J. Physiol. 167:39P–40P (1963).
15. Canfield, P., Lebacq, J., and Marechal, G., J. Physiol. 232:467–483 (1973).
16. Gibbs, C. L., and Seraydarian, K., Proc. Aust. Physiol. Pharmacol. Soc. 3:38 (1972).
17. Podolsky, R. J., and Morales, M. F., J. Biol. Chem. 218:945–959 (1956).
18. Homsher, E., Mommaerts, W. F. H. M., Ricchiuti, N. V., and Wallner, A., J. Physiol. 220:601–625 (1972).
19. Hasselbach, W., and Makinose, M., Biochem. Z. 333:518–528 (1961).
20. Hill, D. K., J. Physiol. 164:31 (1962).
21. Boyer, P. D., in "The Enzymes," 2nd edition, P. D. Boyer, H. Lardy, and K. Myrbäck (Eds.). Academic Press, New York, pp. 577–580 (1959).