J. Physiol. (1983), **345**, *pp.* 167–173
Printed in Great Britain

THE EFFECT OF CIRCULATORY OCCLUSION ON THE GLYCOGEN PHOSPHORYLASE–SYNTHETASE SYSTEM IN HUMAN SKELETAL MUSCLE

BY DIMITRIOS CHASIOTIS AND ERIC HULTMAN

From the Department of Clinical Chemistry II, the Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge, Sweden

(*Received 10 February 1983*)

SUMMARY

1. The effect of circulatory occlusion upon the glycogen phosphorylase–synthetase system in intact human muscle at rest has been investigated using the needle biopsy technique.

2. The fraction of phosphorylase in the *a* form was 26% before occlusion and decreased to 9% after 40 min of occlusion.

3. Synthetase I activity was unchanged during occlusion.

4. After 40 min of occlusion the content of phosphocreatine was decreased by 40%, with a corresponding increase in creatine and inorganic phosphate (P_i).

5. The observed glycogenolytic rate increased during occlusion up to 0·8 mmol glycosyl units kg^{-1} dry muscle min^{-1}.

6. An intracellular P_i concentration at rest of 2·0 mmol l^{-1} was calculated from the activities of phosphorylase *a* and synthetase I assuming that under these conditions they are equal.

7. It is concluded that the glycogenolytic rate during occlusion is a function of both the fraction of phosphorylase in the *a* form and the availability of P_i at the active site of the enzyme.

INTRODUCTION

Occlusion of the circulation to the leg converts the muscle tissue into a closed biological system and limits the chemical events to those which can occur anaerobically. In this case, the main energy sources are the intramuscular content of ATP, phosphocreatine (PCr) and glycogen. The rate of glycogenolysis in this situation can be calculated from the accumulation of hexosemonophosphates and lactate.

Previous studies in animals (Bollman & Flock, 1944; Imai, Riley & Berne, 1964) and in humans (Harris, Hultman, Kaijser & Nordesjö, 1975; Larsson & Hultman, 1979; Cresshull, Dawson, Edwards, Gadian, Gordon, Radda, Shaw & Wilkie, 1981; Harris, 1981) have shown that the muscle content of PCr decreases during occlusion, with a corresponding increase in creatine (Cr) and inorganic phosphate (P_i). P_i is one of the substrates of phosphorylase and it has been suggested by Chasiotis, Sahlin & Hultman (1982, 1983*c*) that the availability of P_i is one of the main factors regulating

the rate of glycogenolysis *in vivo*. However, despite the increased content of P_i within the muscle in the occlusion studies mentioned above, the rate of glycogenolysis was low as judged from the accumulation of hexose monophosphates and lactate.

The present study was undertaken to investigate the effect of circulatory occlusion upon the phosphorylase–synthetase system and its relation to the rate of glycogenolysis *in vivo*.

MATERIALS AND METHODS

Materials. Three healthy volunteers (one female and two males) aged 22, 25 and 30 years and of normal body weight and height participated in the present study. Their training status was not investigated in any detail but none of the subjects could be classified as athletes. The purpose and the nature of the study were explained before their voluntary consent was obtained. This study is part of a project which has been approved by the Ethical Committee of the Karolinska Institute, Stockholm, Sweden.

Experimental procedure. The blood circulation to the subject's leg was occluded by means of a tourniquet placed around the upper part of the thigh and inflated to 250 mmHg. The occlusion was continued for 30–40 min. Muscle tissue was obtained by the needle biopsy technique (Bergström, 1962) from the lateral portion of quadriceps femoris muscle. Samples were taken before occlusion and after 1, 20, 30 and 40 min of occlusion. Immediately after withdrawal, the biopsy needle was immersed in liquid Freon (dichlorodifluoromethane) maintained at its melting point (−150 °C) with liquid nitrogen. The time delay between immersion of the muscle and freezing was between 2 and 3 s. The frozen muscle samples were stored in liquid nitrogen, freeze-dried and thereafter stored at −80 °C until analysed.

Analytical methods. The freeze-dried samples were dissected free from blood, fat and all visible connective tissue, pulverized and divided into two parts. One part was used for the determination of the enzyme activities and the other for determination of metabolites. Homogenates of muscle tissue for assay of glycogen phosphorylase and synthetase were prepared as previously described by Chasiotis *et al.* (1982). The same homogenate was used for assay of glycogen and phosphorylase and synthetase.

Glycogen phosphorylase was assayed at 35 °C and pH 7·0 in the direction of glycogen breakdown using a method adapted from Holmes & Mansour (1968) as previously described by Chasiotis *et al.* (1982). The reaction was started by addition of AMP-free glycogen and formation of hexosemonophosphates was measured enzymically according to Harris, Hultman & Nordesjö (1974). Values are reported as mmol glycosyl units kg^{-1} dry muscle min^{-1}.

Glycogen synthetase was assayed at 35 °C and pH 7·0 in the direction of glycogen synthesis using a method adapted from Kornfeld & Brown (1962) as previously described by Chasiotis *et al.* (1982). The reaction was started by addition of UDP-glucose, and formation of UDP was measured enzymically according to Danforth (1965). Values are reported as mmol glycosyl units kg^{-1} dry muscle min^{-1}.

The muscle contents of ATP, PCr, Cr, glucose-6-phosphate, glucose-1-phosphate, fructose-6-phosphate and lactate were analysed in perchloric acid extracts of freeze-dried muscle by enzymic methods as described by Harris *et al.* (1974). P_i was analysed enzymically as described by Gawehn (1974).

RESULTS

The subjects reported no pain or discomfort during the first 30 min of occlusion except for a subjective feeling of numbness in the leg. After 30 min the subjects complained of increasing discomfort, but this disappeared completely after release of the tourniquet.

The activity of total phosphorylase ($a+b$) and synthetase (I + D) was unchanged during occlusion. Phosphorylase a activity before occlusion was 10·6 (9·5 ± 11·5) mmol

glycosyl units kg^{-1} dry muscle min^{-1} (Table 1), which is in accordance with previous studies (Chasiotis *et al.* 1982, 1983*c*; Chasiotis, Hultman & Sahlin, 1983*b*; Chasiotis, Brandt, Harris & Hultman, 1983*a*). The activity of the *a* form decreased continuously during occlusion and after 40 min was only 4·0 mmol glycosyl units kg^{-1} dry muscle min^{-1} (Table 1). Synthetase I activity was unchanged during occlusion (Table 1).

TABLE 1. Effect of occlusion on enzyme activities in three subjects

		Occlusion			
	Rest	1 min	20 min	30 min	40 min
Phosphorylase *a* + *b*	86·7 (81·2–96·4)	84·8 (74·3–91·1)	85·1 (80·3–89·5)	84·5 (78·4–86·0)	83·1 (76·3–90·0)
Phosphorylase *a*	10·6 (9·5–11·5)	10·1 (8·7–12·4)	7·0 (5·0–8·0)	5·4 (4·0–7·0)	4·0 (3·6–4·3)
Synthetase I + D	9·1 (8·0–9·6)	8·6 (8·0–9·2)	7·5 (6·5–8·3)	8·1 (6·8–8·9)	7·1 (6·8–7·5)
Synthetase I	2·5 (1·9–2·8)	2·3 (1·9–2·5)	2·3 (1·8–2·5)	2·7 (2·4–3·2)	2·5 (2·3–2·7)
$V_{max(a)} : V_{max(a+b)}$	0·26 (0·20–0·29)	0·25 (0·20–0·34)	0·17 (0·13–0·21)	0·13 (0·10–0·19)	0·09 (0·08–0·10)
Estimated net glycogenolysis (phosphorylase *a* − synthetase I)	8·1 (6·7–9·6)	7·9 (6·8–10·5)	4·7 (2·7–7·0)	2·7 (1·4–5·3)	1·5 (1·0–2·0)

Values are given as mean (range). Enzyme activities and net glycogenolysis are given as mmol glycosyl units kg^{-1} dry muscle min^{-1}. $V_{max(a)}$ and $V_{max(a+b)}$ have been calculated from phosphorylase activities measured at 11 mmol l^{-1} P_i using a K_m of 26·2 and 6·8 mmol l^{-1} for *a* and *a* + *b* respectively (Chasiotis *et al.* 1982, 1983*c*). Net glycogenolysis is calculated from measured phosphorylase *a* and synthetase I activities assuming that phosphorylase *b* and synthetase I are inactive in muscle at rest and during occlusion.

The activities of phosphorylase presented in Table 1 were measured at a P_i concentration of 11 mmol l^{-1}. The K_m values of phosphorylase *a* and *a* + *b* for P_i have previously been determined to be 26·2 and 6·8 mmol l^{-1} respectively in resting muscle (Chasiotis *et al.* 1982, 1983*c*). From the measurements of phosphorylase *a* and *a* + *b* activity at 11 mmol l^{-1} P_i and the values of K_m, the ratio $V_{max(a)} : V_{max(a+b)}$ has been calculated (Table 1). This ratio may be used as an estimate of the proportion of phosphorylase molecules in the *a* form (Chasiotis *et al.* 1982). Accordingly the *a* form accounted for 25·8 % of total phosphorylase before occlusion, the proportion decreasing continuously during occlusion to 9 % after 40 min.

During the 40 min of occlusion PCr decreased by 40 %, this decrease being paralleled by corresponding increases in Cr and P_i (Table 2). The average rate of PCr breakdown was 0·7 mmol kg^{-1} dry muscle min^{-1}. The muscle content of ATP was essentially unchanged during occlusion. Hexosemonophosphates (mainly glucose-6-phosphate) increased 2·5-fold and lactate 7-fold during the occlusion period (Table 2). The rates of glycogenolysis calculated from the accumulation of hexosemonophosphates and lactate between 0–20, 20–30 and 30–40 min were 0·24 (0·22–0·27), 0·50 (0·28–0·75) and 0·75 (0·44–1·06) mmol glycosyl units kg^{-1} dry muscle min^{-1}, respectively (Table 2).

TABLE 2. Effect of occlusion on the muscle content of energy metabolites, hexosemonophosphates, lactate and inorganic phosphate

		Occlusion		
	Rest	20 min	30 min	40 min
PCr	67·1 (64·0–69·2)	45·7 (38·2–55·7)	43·7 (35·4–45·4)	40·2 (37·9–42·4)
Cr	52·1 (51·3–54·9)	76·7 (68·9–86·2)	77·0 (73·0 86·7)	72·5 (55·0–97·0)
ATP	23·6 (23·0–26·0)	22·5 (21·8–23·6)	22·9 (22·2–23·0)	24·5 (23·4–25·7)
Hexosemono-phosphates	2·7 (1·5–3·3)	4·4 (3·9–4·6)	5·6 (4·8–6·4)	6·3 (6·3–6·3)
Lactate	4·1 (3·5–5·0)	11·5 (11·3–11·9)	16·4 (14·3–17·4)	30·5 (26·3–34·7)
P_i	49·6 (45·0–53·2)	64·8 (62·4–67·4)	62·5 (59·9–64·7)	74·0 (74·0–74·1)
		0–20 min	20–30 min	30–40 min
Glycogenolytic rate	—	0·24 (0·22–0·27)	0·50 (0·28–0·75)	0·75 (0·44–1·06)

Values are given as mmol kg^{-1} dry muscle. Glycogenolytic rate was calculated from the accumulation of hexosemonophosphates and lactate, and is given as mmol glycosyl units kg^{-1} dry muscle min^{-1}.

DISCUSSION

The main finding in the present study was a decrease in the fraction of phosphorylase *a* following occlusion of the circulation. The results are in contrast to previous studies in rat heart (Morgan & Parmeggiani, 1964) and in brain tissue (Lowry, Passonneau, Hasselberger & Schulz, 1964) in which an increase in phosphorylase *a* was observed during ischaemia.

We are unable at present to explain the mechanism by which the fall in phosphorylase *a* in muscle is brought about. One possibility could be that it results from the muscle cells being shielded during occlusion from the effects of blood-borne stimuli including adrenaline. In previous studies we have shown that the fraction of phosphorylase in the *a* form increases from 22·5 to 90% during adrenaline infusion (Chasiotis *et al.* 1983*c*) but decreases after β-blockade at rest from 22·5 to 17·2% (Chasiotis *et al.* 1983*a*). These changes occur in parallel with those in cyclic AMP and we would propose that following occlusion there is a steady decline in the muscle content of this metabolite. A similar argument might also explain why in isolated muscle of animals very low levels of phosphorylase *a* (2–10%) have been found (Danforth, Helmreich & Cori, 1962; Danforth & Lyon, 1964) compared with values of 10–30% determined in muscle *in situ* (Drummond, Harwood & Powell, 1969; Conlee, McLane, Rennie, Winder & Holloszy, 1979).

It could be argued that the high levels of phosphorylase *a* found at rest in the present study are an artifact resulting from release of calcium ions during the biopsy procedure rather than a reflexion of cyclic AMP stimulation. The decrease in phosphorylase *a* during occlusion, however, shows that calcium-mediated transformation during sampling and analysis can largely be excluded, since the effect should be the same as at rest.

As mentioned above, synthetase I was unchanged during occlusion. If it is assumed that phosphorylase *b* and synthetase D are inactive in muscle at rest and during occlusion, then estimates of the net glycogenolytic rate may be calculated from the activities of phosphorylase *a* minus those of synthetase I. We have assumed

synthetase I to be fully active. From the measurement of *a* activity at 11 mmol l^{-1} P_i, the estimated rate of glycogenolysis at rest is 10·6 − 2·5 = 8·1 mmol glycosyl units kg^{-1} dry muscle min^{-1}. At this rate, however, the intramuscular glycogen store would be depleted within 50 min, and clearly this is not the case. Almost certainly the estimates of phosphorylase *a* activity used are far in excess of those occurring *in situ*. There are reasons to believe that the true P_i concentration in resting muscle is much lower than that used here to assay phosphorylase *a* activity (i.e. 11 mmol l^{-1} P_i). This P_i value was determined experimentally in perchloric acid extracts of human muscle and is probably too high due to release of P_i from proteins and other molecules during extraction. Recent ^{31}P nuclear magnetic resonance studies have indicated a free P_i concentration in muscle of the order of 1–3 mmol l^{-1} (Dawson, Gadian & Wilkie, 1977; Cresshull *et al.* 1981).

We can, in fact, make an estimate of the intracellular P_i concentration based on the activities of phosphorylase *a* and synthetase I. For this we must first assume a net rate of glycogen synthesis or degradation at rest of zero (which is consistent with the findings of Hultman, 1967), and thus equal activities of phosphorylase *a* and synthetase I. The calculated P_i concentration is that which would support a phosphorylase *a* activity equal to the synthetase I activity at rest (given in Table 1) and is obtained from:

$$\left[P_i\right] = \frac{S_{11} \cdot K_m \cdot V_I}{V_{11} \cdot K_m + S_{11} \cdot V_{11} - V_I \cdot S_{11}},$$

where S_{11} is the P_i concentration of the assay, 11 mmol l^{-1}; K_m for phosphorylase *a* for P_i is (from Table 1) 26·2 mmol l^{-1} P_i; V_{11} is phosphorylase *a* activity at rest at 11 mmol l^{-1} P_i; and V_I is synthetase I activity at rest.

From the values in Table 1:

$$[P_i] = 2{\cdot}0 \text{ mmol } l^{-1}.$$

As discussed in a previous paper (Chasiotis *et al.* 1982) synthetase I at rest is at best only partially active and this would tend to lower the above estimate of P_i concentration. Similarly, residual phosphorylase *b* activity or a lower K_m of phosphorylase *a* for P_i *in vivo*, due to the presence of AMP, would also lower the P_i concentration. Only activity on the part of synthetase D would increase the above estimate of P_i, but the possibility of this seems very remote. Our conclusion therefore is that the true intracellular P_i concentration at rest lies somewhere between 0 and 2 mmol l^{-1}, a range which is in close agreement with the previously mentioned nuclear magnetic resonance studies.

The same type of calculations can be applied to the 20 and 30 min estimates of glycogenolysis. The calculated rates (Table 1) were again higher than the observed rates and clearly reflect an intracellular P_i concentration far removed from that used in the assays of phosphorylase *a*. After 40 min of occlusion, however, calculated rates were of the same order as observed rates, indicating a P_i concentration approaching 11 mmol l^{-1}. This is confirmed if the changes in PCr and hexosemonophosphates presented in Table 2 are examined. Net P_i release (PCr minus hexosemonophosphates) amounted to 7·5 mmol l^{-1} after 40 min of occlusion, to which should be added 2·0 mmol l^{-1}, the supposed concentration at rest.

The possibility that phosphorylase *b* could be activated by AMP or IMP during occlusion has been considered. At rest the concentration of AMP in the same muscle of man is of the order of 0·03 mmol l^{-1} (Chasiotis *et al.* 1982; Larsson & Hultman, 1979). The maximum AMP content observed after 20 min of occlusion was 0·06 mmol l^{-1} (Harris *et al.* 1975) and after 90–150 min, 0·09 mmol l^{-1} (Larsson & Hultman, 1979). These values are far below the apparent K_m value of phosphorylase *b* for AMP of 1 mmol l^{-1} determined in the presence of physiological concentrations of ATP and glucose-6-phosphate and in the direction of glycogen breakdown (Aragon, Tornheim & Lowenstein, 1980). Even assuming all the AMP to be freely available to phosphorylase *b* these concentrations are unlikely to result in significant activity of this form of the enzyme. From similar considerations there seems little possibility either that IMP has any significant effect on phosphorylase *b*.

Muscle, when stimulated, is one of the most metabolically active tissues in the body, but at rest the turnover rate is low. This is despite nearly a quarter of phosphorylase being in the *a* form, its activity being limited by a low P_i concentration and opposing glycogen synthetase activity. During occlusion PCr is broken down, the net result being the liberation of P_i. In the absence of further changes the amount released after 20–40 min would be more than sufficient to increase phosphorylase *a* activity several-fold and would result in rates of glycogenolysis far in excess of the modest increase actually seen during occlusion. Two effects, however, combine to prevent this. As shown in the present report, phosphorylase *a* is converted to the *b* form such that after 40 min of occlusion it accounts for only 9% of the total phosphorylase activity. It is concluded that the *b* form is inactive since otherwise no purpose would be served by this conversion; glycogenolysis would continue to rise with P_i to rates far in excess of those observed. Secondly the modest increase in glycogenolysis itself results in the accumulation of hexosemonophosphates, removing part of the P_i liberated as a result of PCr utilization. In this sense the system is self-regulating. As have previous papers (Chasiotis *et al.* 1982, 1983*c*) the present results emphasize the integration of phosphorylase *a*/*b* transformation and P_i as determinants of the glycogenolytic rate in muscle *in vivo*.

We are greatly indebted to Dr Roger C. Harris for his assistance in the preparation of the manuscript and for many helpful ideas, and to Lennart Fahlgren for determination of hexosemonophosphates and lactate. This work was supported by a grant from the Swedish Medical Research Council (02647).

REFERENCES

ARAGON, J. J., TORNHEIM, K. & LOWENSTEIN, J. M. (1980). On a possible role of IMP in the regulation of phosphorylase activity in skeletal muscle. *FEBS Lett., Suppl.* **117**, K56–K66.

BERGSTRÖM, J. (1962). Muscle electrolytes in man, determined by neutron activation analysis on needle biopsy specimens. A study on normal subjects, kidney patients, and patients with chronic diarrhoea. *Scand. J. clin. Lab. Invest.* **68**, 1–110.

BOLLMAN, J. L. & FLOCK, E.-V. (1944). Changes in phosphate of muscle during tourniquet shock. *Am. J. Physiol.* **142**, 290–297.

CHASIOTIS, D., SAHLIN, K. & HULTMAN, E. (1982). Regulation of glycogenolysis in human muscle at rest and during exercise. *J. appl. Physiol.* **53**, 708–715.

CHASIOTIS, D., BRANDT, R., HARRIS, R. C. & HULTMAN, E. (1983*a*). The effects of β-blockade on glycogen metabolism in man during exercise. *Am. J. Physiol.* (in the Press).

CHASIOTIS, D., HULTMAN, E. & SAHLIN, K. (1983*b*). Acidotic depression of cyclic AMP accumulation and phosphorylase *b* to *a* transformation in skeletal muscle of man. *J. Physiol.* **335**, 197–204.

CHASIOTIS, D., SAHLIN, K. & HULTMAN, E. (1983*c*). Regulation of glycogenolysis in human muscle in response to epinephrine infusion. *J. appl. Physiol.* **54**, 45–50.

CONLEE, R. K., MCLANE, J. A., RENNIE, M. J., WINDER, W. W. & HOLLOSZY, J. O. (1979). Reversal of phosphorylase activation in muscle despite continued contractile activity. *Am. J. Physiol.* **237**, R291–R296.

CRESSHULL, I., DAWSON, M. J., EDWARDS, R. H. T., GADIAN, D. G., GORDON, R. E., RADDA, G. K., SHAW, D. & WILKIE, D. R. (1981). Human muscle analysed by ^{31}P nuclear magnetic resonance in intact subjects. *J. Physiol.* **317**, 18*P*.

DANFORTH, W. H. (1965). Glycogen synthetase activity. Interconversion of two forms and control of glycogen synthesis. *J. biol. Chem.* **250**, 588–593.

DANFORTH, W. H., HELMREICH, E. & CORI, C. F. (1962). The effect of contraction and epinephrine on the phosphorylase content of frog sartorius muscle. *Proc. natn. Acad. Sci. U.S.A.* **48**, 1191–1199.

DANFORTH, W. H. & LYON, J. B., JR (1964). Glycogenolysis during tetanic contraction of isolated mouse muscles in the presence and absence of phosphorylase *a*. *J. biol. Chem.* **239**, 4047–4050.

DAWSON, M. J., GADIAN, D. G. & WILKIE, D. R. (1977). Contraction and recovery of living muscles studied by ^{31}P nuclear magnetic resonance. *J. Physiol.* **267**, 703–735.

DRUMMOND, G. I., HARWOOD, J. P. & POWELL, C. A. (1969). Studies on the activation of phosphorylase in skeletal muscle by contraction and by epinephrine. *J. biol. Chem.* **244**, 4235–4240.

GAWEHN, K. (1974). UV-spectrometric methods. In *Methods of Enzymatic Analysis*, ed. BERGMEYER, E., pp. 2234–2238. New York: Academic Press.

HARRIS, R. C. (1981). Muscle energy metabolism in man in response to isometric contraction. A biopsy study. M.Sc. thesis, University of Wales.

HARRIS, R. C., HULTMAN, E., KAIJSER, L. & NORDESJÖ, L.-O. (1975). The effect of circulatory occlusion on isometric exercise capacity and energy metabolism of the quadriceps muscle in man. *Scand. J. clin. Lab. Invest.* **35**, 87–95.

HARRIS, R. C., HULTMAN, E. & NORDESJÖ, L.-O. (1974). Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variances of values. *Scand. J. clin. Lab. Invest.* **33**, 109–119.

HOLMES, P. E. & MANSOUR, T. E. (1968). Glucose as a regulator of glycogen phosphorylase and glycogen levels. *Biochim. biophys. Acta* **156**, 266–274.

HULTMAN, E. (1967). Muscle glycogen in man determined in needle biopsy specimens. *Scand. J. clin. Lab. Invest.* **19**, 209–217.

IMAI, E., RILEY, A. L. & BERNE, R. M. (1964). Effects of ischaemia on adenine nucleotides in cardiac and skeletal muscle. *Circulation Res.* **15**, 443–450.

KORNFELD, R. & BROWN, D. H. (1962). Preparation and properties of uridine diphosphate glucose–glycogen transglucosylase. *J. biol. Chem.* **237**, 1772–1777.

LARSSON, J. & HULTMAN, E. (1979). The effect of long-term arterial occlusion on energy metabolism of the human quadriceps muscle. *Scand. J. clin. Lab. Invest.* **39**, 257–264.

LOWRY, D. H., PASSONNEAU, J. V., HASSELBERGER, F. X. & SCHULZ, D. W. (1964). Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. *J. biol. Chem.* **239**, 18–30.

MORGAN, H. E. & PARMEGGIANI, A. (1964). Regulations in glycogenolysis in muscle. *J. biol. Chem.* **238**, 254–272.