PHARMACOLOGICAL REVIEWS
Vol. 18, No. 1, Part I
Copyright © 1966 by The Williams & Wilkins Co.
Printed in U.S.A.

E. THE ACTIVATION OF GLYCOLYSIS IN FROG SARTORIUS MUSCLE BY EPINEPHRINE[1]

ERNST HELMREICH AND CARL F. CORI

Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri

Lesser (11) showed in 1920 that treatment of isolated frog muscle with epinephrine (E) increases the rate of glycogenolysis. In 1934 Hegnauer and G. T. Cori (8) demonstrated that glycogenolysis in the E-treated anaerobic frog muscle results in a large increase in hexosephosphate and a corresponding fall in inorganic P with relatively little change in lactate production. In 1950 O. and E. Walaas (21) observed that addition of a few micrograms of E to the isolated rat diaphragm incubated in the presence of glucose caused a significant increase in hexosephosphate and lactate and a net loss of glycogen instead of glycogen deposition. In 1951 Sutherland and Cori (19) showed that homogenates prepared from liver slices preincubated with E contained more active phosphorylase than those not exposed to the hormone. The same observations were made by Sutherland (18) with isolated rat diaphragm. His work led to the important discovery of cyclic 3′,5′-AMP as the mediator of the rise in phosphorylase activity in response to E treatment (17).

Development of methods for the rapid arrest of enzymatic reactions in living muscle coupled with improved analytical techniques made it possible to reinvestigate in greater detail the mechanism of activation of glycolytic enzymes in frog muscle in response to E. Some of the more recent results will be presented in this paper.

METHODS

The sartorius muscle of winter frogs (*Rana pipiens*) was used for these experiments. All incubations were carried out in the absence of glucose in frog Ringer's bicarbonate solutions gassed with 95% Argon, 5% CO_2. In order to avoid transition effects and to insure complete anaerobiosis, the muscles were incubated usually at 20° C for 25 min prior to the addition of E. In the course of the experiment muscles were transferred to fresh medium every 20 min in order to measure the efflux of lactate. The amount found in the medium is referred to as extracellular lactate. Based on measurements of extracellular space and of total tissue water, concentrations are expressed in terms of micromoles per milliliter of intracellular water. The incubations were terminated by plunging the muscles into isopentane cooled to −170°C. The media were likewise frozen immediately. Procedural details have been published (9).

Analyses for lactate and glucose-6-P were carried out on the same muscle using chemical and enzymatic procedures (9). Inorganic P was determined as described previously (9). Methods for the extraction of phosphorylase at tem-

[1] This work was supported in part by research grants AM-6830 and AM-9242 from the National Institutes of Health, U. S. Public Health Service.

peratures below 0° C and methods for the determination of phosphorylase *b* and *a* activity have likewise been published (4). Epinephrine hydrochloride was obtained from Parke, Davis and Company and stored frozen as 1×10^{-4} M solution containing 1×10^{-2} M ascorbate. Except where stated otherwise, E was used in a concentration of 5×10^{-7}M (*ca.* 1 in 10 million) in frog Ringer's solution containing 1×10^{-3} M ascorbate, pH 7.0. Dichloroisoproterenol (DCI) was a gift of Dr. I. H. Slater, Eli Lilly Co.

RESULTS

The effect of epinephrine on the glycolytic system in frog muscle. The effect of E on the rate of glycogenolysis in the anaerobic frog sartorius at 20° C is shown in figure 1. It was assumed that "glycogen lost" was equal to one half lactate + glucose-1-P + glucose-6-P + fructose-6-P. Glucose-1-P and fructose-6-P were assumed to be 0.05 and 0.2 times the concentration of glucose-6-P, respectively (15). The amount of fructose-1,6-diphosphate present in the E-treated muscle or in the anaerobic control muscle was negligible (15). In the 10- to 30-min period of incubation with epinephrine the rate of glycogenolysis of frog muscle is increased about 10-fold over the basal rate. This increase in rate is preceded by a lag period of 3 to 4 min duration. E action results in a sharp rise in glucose-6-P [and, as shown in other experiments, of fructose-6-P (15)] without a concomitant change in the rate of lactate formation. Only after 30 min does the rate of lactate formation begin to increase. It then attains a rate of 0.04 μmoles per ml per min, which is only 4 times faster than the basal rate. A steady state between formation of glucose-6-P and its removal is not established since glu-

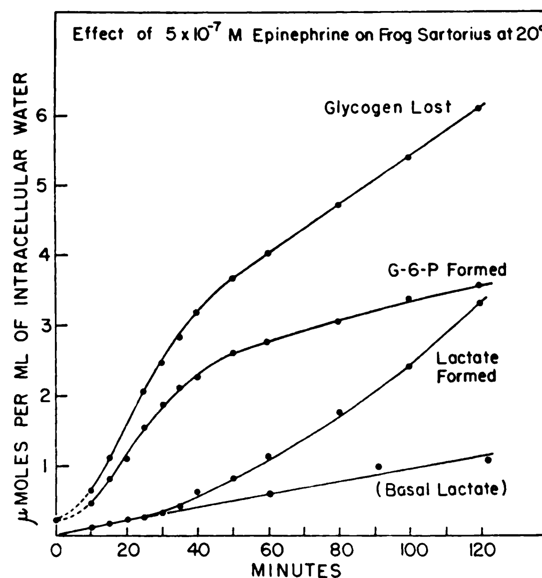

FIG. 1. Effect of epinephrine on glycogenolysis in frog muscle. (From J. biol. Chem. **239**: 3139, 1964.) For calculation of the amount of "Glycogen Lost," see text.

TABLE 1

Comparison of effects of electrical stimulation and of E on glycolysis of frog muscle

Electrical Stimulation (30 min Duration)	Increase in Glucose-6-P over Control	Decrease in Inorganic P over Control	Increase in Lactate over Control
Shocks/min		*μmoles/ml*	
3	0.2		6.7
6	0.2		9.8
12	0.2		14.9
18	0.3		20.6
24	0.6		26.1
5×10^{-7} M E			
30 min	1.7	1.3	0
60 min	2.6	2.8	0.8
120 min	3.4	3.6	3.0

The experiments were carried out anaerobically at 20°C, without added glucose.

TABLE 2

Effect of dichloroisoproterenol on the formation of glucose-6-P in E-treated frog muscle

Additions	Glucose-6-P
M	*μmoles/ml*
None	0.2 ± 0.08
DCI (5×10^{-5})	0.25 ± 0.07
E (5×10^{-7})	2.71 ± 0.44
E (5×10^{-7}) + DCI (5×10^{-5})	0.13 ± 0.01

The muscles were incubated anaerobically for 1 hr at 30°C with the additions as noted. Mean value and standard error of the mean of five experiments are given. Summer frogs were used in these experiments.

cose-6-P is still rising at the end of the incubation period, although at a slower rate. This is in marked contrast to the effect of electrical stimulation. As shown in table 1, electrical stimulation causes a much greater increase in lactate formation without much change in the concentration of glucose-6-P up to 24 shocks per min. It appears that enzymatic rates are not as well synchronized in muscle during E action as they are during stimulation (9).

Activation of the phosphorylase system in the E-treated frog muscle. When one compares the decrease in inorganic P produced by E with the increase in glucose-6-P, it becomes apparent that at each time point there is a corresponding change in the concentrations of these two compounds (table 1). The reaction sequence is: glycogen + inorganic P → glucose-1-P → glucose-6-P. The first reaction is catalyzed by phosphorylase. Dichloroisoproterenol (DCI), an analogue of E, completely abolishes the rise in glucose-6-P resulting from E action (table 2). This suggests that the effect of E on glycogenolysis in frog muscle is mediated through increased phosphorylase activity. The conversion of phosphorylase *b* to *a* during E action or during stimulation is one possible mechanism for the activation of

the phosphorylase system. It is therefore of interest that DCI blocks the rise in phosphorylase *a* produced by E but has no effect on the increase produced by stimulation (table 3). In the former case the rise in cyclic 3′,5′-AMP, an activator of phosphorylase *b* kinase, is prevented by DCI (7, 14). Since Posner *et al.* (16) have shown that the concentration of cyclic 3′,5′-AMP does not increase in the stimulated muscle, it seems likely that the mechanism of activation of phosphorylase *b* kinase is different in the two cases.

In table 4 the rise and fall of phosphorylase *a* in the E-treated muscle and in the muscle stimulated tetanically are compared. The rise of phosphorylase *a* in the E-treated muscle is rather slow, but this is also the case when E is injected intracardially in the intact frog (16). The decrease in phosphorylase *a* after stimulation has been shown to be a first-order process catalyzed by phosphorylase phosphatase, now no longer opposed by the action of phosphorylase kinase (4). It might therefore be inferred that the slow reversal of the E effect in the isolated frog muscle is related to the slow release of the hormone from the receptor site and hence to the persistence of elevated levels of cyclic 3′,5′-AMP. The fact that inclusion of DCI in the wash fluid greatly accelerates the fall in phosphorylase *a* supports this assumption (table 4).

Effect of epinephrine on muscles stimulated electrically. When frog sartorius muscle is stimulated by single shocks at rates from 2 to 8 per sec, the phosphorylase *a* content rises to a plateau which is proportional to the frequency of stimulation (5). The rise in phosphorylase *a* is preceded by a lag period which at 2

TABLE 3

Differential effect of DCI on the formation of phosphorylase a in frog muscles treated with E or stimulated electrically

Conditions	Phosphorylase *a* in Percent of Total Phosphorylase	
	−DCI	+DCI
E 1.1 × 10^{-6} M	64	3
Muscle contraction	84	72

Paired muscles from a single frog were incubated anaerobically at 30°C for 30 min in the presence or absence of 1 × 10^{-4} M DCI. E was then added and the incubation was continued for an additional 30 min. The muscles to be stimulated were also preincubated with or without DCI. They were fixed after a 2 or 4 sec tetanus.

TABLE 4

Comparison of effects of stimulation and E on rise and fall of phosphorylase a in frog muscle

Rise in Phosphorylase *a*		Fall in Phosphorylase *a*	
Conditions	t ½ (sec)	Conditions	t ½ (sec)
Tetanic stimulation	0.7	Rest	12
E (1.1 × 10^{-6} M)	370	Washout	660
		Washout + DCI (1.1 × 10^{-4} M)	150

All experiments were carried out anaerobically at 30°C.

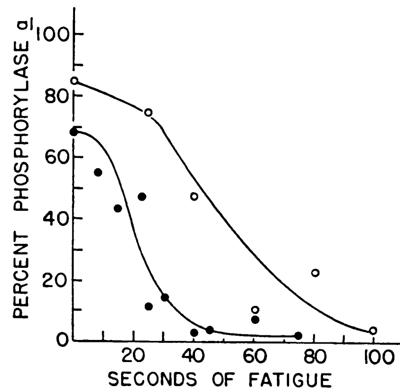

FIG. 2. Effect of fatigue on phosphorylase *a* content of frog muscle. (From J. biol. Chem. 239: 3133, 1964.) The design of the experiment is explained in the text.

shocks per sec is about 25 sec. With increasing frequencies of stimulation the lag period becomes progressively shorter. When the muscle is first exposed for 30 min to subthreshold concentrations of E (1×10^{-8} to 7×10^{-8} M), which *per se* produce no rise in phosphorylase *a*, the lag period during stimulation at 2 shocks per sec is completely abolished and the plateau to which phosphorylase *a* rises is higher (5). The lag period probably reflects a delay in the onset of activity of the phosphorylase *b* kinase. A similar delay in the onset of activity is observed *in vitro*, when the utilization of ATP with phosphorylase *b* as substrate is used as a measure of the rate of the kinase reaction (5). This rules out the possibility that the delay is related to some configurational change of phosphorylase after the kinase has acted.

The facilitation by E of the $b \rightarrow a$ conversion in stimulated muscle may be related to the effect of E on contractility of skeletal muscle. As is well known, this effect is particularly prominent in fatigued muscle. G. T. Cori and Illingworth (3) have shown that when rat gastrocnemius muscle was stimulated to fatigue by repeated short tetani, the phosphorylase *a* content fell to very low values. Previous injection of the rat with E prevented the complete disappearance of phosphorylase *a* during stimulation to fatigue, and injection of E after fatigue had set in hastened the recovery of phosphorylase *a*. This problem has been reinvestigated with the rapid fixation technique (4), which was not available in the earlier experiments. In figure 2, the muscles were first stimulated at 6 shocks per sec for periods of time recorded on the abscissae. After 5 min of recovery to allow the phosphorylase *a* to revert to the *b* form, a second train of single shocks at 6 per sec for 20 sec was given to each muscle. The ordinates show the level of phosphorylase *a* attained at the end of the second period of stimulation. It can be seen that the more fatigued the muscle was, the less it was capable of responding with an increase in phosphorylase *a* during stimulation (lower curve). Prior exposure of the muscles to E (upper curve) resulted in increased resistance to fatigue as measured by their ability to form phosphorylase *a*.

The importance of phosphorylase *a* for rapid glycogenolysis is shown in an

TABLE 5

Effect of electrical stimulation on lactate formation in frog muscle in the presence and absence of E

Frequency and Duration of Stimulation	Lactate Formed during Stimulation in the Absence of E		Lactate Formed during Stimulation in the Presence of E	
	Extracellular	Total	Extracellular	Total
	µmoles/ml			
3 S. P. M. for 30 min		6.30		6.42
6 S. P. M. for 10 min	0.38		0.24	
6 S. P. M. for 20 min	1.05	7.21	0.84	6.33
6 S. P. M. for 30 min	2.12	9.57	2.27	9.80
12 S. P. M. for 10 min	0.46		0.43	
12 S. P. M. for 20 min	1.53		1.57	
12 S. P. M. for 30 min	3.42	14.80	3.46	12.98

Incubations were anaerobic at 20°C. E when added was 5×10^{-7} M. S. P. M. refers to shocks per minute.

experiment carried out by Danforth and Lyon (6). After 1 sec of stimulation, the muscle of normal mice (C57 strain), containing about 50% phosphorylase *a*, has a more rapid and extensive glycogen breakdown than does the muscle of a strain of mice (I strain) lacking phosphorylase *b* kinase. It seems reasonable to suggest that the effect of E in delaying muscle fatigue is related to the ability of muscle to maintain its phosphorylase *a* level, since this would make possible a more rapid supply of energy to the working muscle.

Phosphofructokinase activity of frog muscle during epinephrine action. From the experiment in figure 1, it has been deduced that in isolated frog muscle treated with E there is initially a much greater increase in phosphorylase than in phosphofructokinase activity. As a result the concentrations of glucose-6-P and fructose-6-P rise, whereas that of fructose-1,6-diP remains very low (about 0.02 µmoles/ml (15)). During 30 min of incubation with E the concentration of hexose monophosphate has risen to about 10 times the values found in control muscles, but an increase in lactate formation has not yet set in. During stimulation of muscle a similar rise in hexose monophosphate is associated with a large increase in lactate formation (*cf.* table 1). These results raised the question whether E might actually cause inhibition of phosphofructokinase activity in muscle. When frog muscle previously treated with E was given a short tetanus there was an immediate and substantial rise in fructose-1,6-diP up to values of about 1 µmole per ml intracellular water (15). Although this experiment indicated responsiveness of phosphofructokinase to activation by stimulation, it would not rule out some degree of inhibition. A more rigorous test for inhibition is provided by the experiments in table 5, where muscles were stimulated with single shocks at relatively slow rates. It can be seen that E did not have an inhibitory effect on lactate formation in these stimulated muscles. It is therefore not inhibition but rather lack of activation which characterizes the effect of E on phosphofructokinase activity, at least in amphibian muscle. In mammalian mus-

cle hexose monophosphate is also markedly increased as the result of E action, but at the same time there is a greatly increased lactate production (see 2).

DISCUSSION AND SUMMARY

The sequence of events set in motion through the activation of adenyl cyclase by E is: increase in cyclic 3′,5′-AMP → activation of phosphorylase *b* kinase → increase in phosphorylase *a* → increase in glycogen breakdown. Since cyclic 3′,5′-AMP is destroyed by a phosphodiesterase, its actual level in the tissues will depend on the relative rates of two opposing reactions, catalyzed by cyclase and diesterase (1, 20). Posner *et al.* (16) have shown that the concentration of cyclic 3′,5′-AMP increases in frog muscle after E administration. Concentrations of the order of 1×10^{-7} to 1×10^{-6} M activate the phosphorylase *b* kinase of muscle both *in vivo* and *in vitro* (10, 16). Phosphofructokinase, on the other hand, is activated *in vitro* by concentrations of cyclic 3′,5′-AMP so much higher than are present in muscle that one wonders whether this activation is of physiological importance (13). In the isolated frog sartorius incubated at 20° C in 5×10^{-7} M E a rise in phosphorylase *a* can be demonstrated in less than 5 min and this is followed after a lag period of a few minutes by a rise in glucose-6-P, but an increased lactate production through activation of phosphofructokinase does not take place until after 30 min of incubation. Stimulation of a muscle during incubation in E at frequencies as low as 3 shocks per minute, results in an immediate rise in lactate production. Thus, there is no evidence that E has a specific activating effect on phosphofructokinase. Özand and Narahara (15) found that addition of glucagon-free insulin to one of a pair of muscles incubated in E resulted in a smaller accumulation of glucose-6-P and in a larger production of lactate; this result suggests that insulin enhanced the activity of phosphofructokinase. At present it is not definitely known by what mechanism electrical stimulation or insulin increases phosphofructokinase activity in muscle.

That the problem of activation of glycolytic enzymes *in vivo* is complex is shown by the fact that according to Lyon and Porter (12) muscle of mice lacking phosphorylase *b* kinase respond to the injection of epinephrine with increased glycogen breakdown. This suggests that E can increase phosphorylase *b* activity in an as yet undetermined manner. It would seem that the mechanism of regulation of enzyme activity by hormones requires further study.

REFERENCES

1. Butcher, R. W. and Sutherland, E. W.: Adenosine 3′,5′-phosphate in biological materials. I. Purification and properties of cyclic 3′,5′-nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3′,5′-phosphate in human urine. J. biol. Chem. **237**: 1244–1250, 1962.
2. Cori, C. F.: Mammalian carbohydrate metabolism. Physiol. Rev. **11**: 143–275, 1931.
3. Cori, G. T. and Illingworth, B.: The effect of epinephrine and other glycogenolytic agents on the phosphorylase *a* content of muscle. Biochim. biophys. Acta, **21**: 105–110, 1956.
4. Danforth, W. H., Helmreich, E. and Cori, C. F.: The effect of contraction and of epinephrine on the phosphorylase activity of frog sartorius muscle. Proc. nat. Acad. Sci., Wash. **48**: 1191–1199, 1962.
5. Danforth, W. H. and Helmreich, E.: Regulation of glycolysis in muscle. I. The conversion of phosphorylase *b* to phosphorylase *a* in frog sartorius muscle. J. biol. Chem. **239**: 3133–3138, 1964.
6. Danforth, W. H. and Lyon, J. B., Jr.: Glycogenolysis during tetanic contraction of isolated mouse muscles in the presence and absence of phosphorylase *a*. J. biol. Chem. **239**: 4047–4050, 1964.
7. Hammermeister, K. E., Yunis, A. A. and Krebs, E. G.: Studies on phosphorylase activation in the heart. J. biol. Chem. **240**: 986–991, 1965.

8. Hegnauer, A. H. and Cori, G. T.: The influence of epinephrine on chemical changes in isolated frog muscle. J. biol. Chem. **105**: 691–703, 1934.
9. Karpatkin, S., Helmreich, E. and Cori, C. F.: Regulation of glycolysis in muscle. II. Effect of stimulation and epinephrine in isolated frog sartorius muscle. J. biol. Chem. **239**: 3139–3145, 1964.
10. Krebs, E. G., Love, D. S., Bratvold, G. E., Trayser, K. A., Meyer, W. L. and Fischer, E. H.: Purification and properties of rabbit skeletal muscle phosphorylase *b* kinase. Biochemistry **3**: 1022–1033, 1964.
11. Lesser, E. J.: Der Mechanismus der Zuckermobilisierung durch das Adrenalin. Biochem. Z. **102**: 304–319, 1920.
12. Lyon, J. B., Jr. and Porter, J.: The relation of phosphorylase to glycogenolysis in skeletal muscle and heart of mice. J. biol. Chem. **238**: 1–11, 1963.
13. Mansour, T. E.: Studies on heart phosphofructokinase: Purification, inhibition and activation. J. biol. Chem. **238**: 2285–2292, 1963.
14. Murad, F., Chi, Y.-M., Rall, T. W. and Sutherland, E. W.: Adenyl cyclase. III. The effect of catecholamines and choline esters on the formation of adenosine 3′,5′-phosphate by preparations from cardiac muscle and liver. J. biol. Chem. **237**: 1233–1238, 1962.
15. Özand, P. and Narahara, H. T.: Regulation of glycolysis in muscle. III. Influence of insulin, epinephrine, and contraction on phosphofructokinase activity in frog skeletal muscle. J. biol. Chem. **239**: 3146–3152, 1964.
16. Posner, J. B., Stern, R. and Krebs, E. G.: Effects of electrical stimulation and epinephrine on muscle phosphorylase, phosphorylase *b* kinase, and adenosine 3′,5′-phosphate. J. biol. Chem. **240**: 982–985, 1965.
17. Rall, T. W. and Sutherland, E. W.: Formation of a cyclic adenine ribonucleotide by tissue particles. J. biol. Chem. **232**: 1065–1076, 1958.
18. Sutherland, E. W.: The effect of the hyperglycemic factor and epinephrine on liver and muscle phosphorylase. In: Phosphorus Metabolism, ed. by W. D. McElroy and B. Glass, Vol. I, pp. 53–61, The Johns Hopkins Press, Baltimore, 1951.
19. Sutherland, E. W. and Cori, C. F.: Effect of hyperglycemic-glycogenolytic factor and epinephrine on liver phosphorylase. J. biol. Chem. **188**: 531–543, 1951.
20. Sutherland, E. W., Rall, T. W. and Menon, T.: Adenyl cyclase. I. Distribution, preparation, and properties. J. biol. Chem. **237**: 1220–1227, 1962.
21. Walaas, O. and Walaas, E.: Effect of epinephrine on rat diaphragm. J. biol. Chem. **187**: 769–776, 1950.