ACTIVE AND INACTIVE FORMS OF PYRUVATEDEHYDROGENASE IN SKELETAL MUSCLE AS RELATED TO THE METABOLIC AND FUNCTIONAL STATE OF THE MUSCLE CELL

G. HENNIG, G. LÖFFLER and O. H. WIELAND
Forschergruppe Diabetes und Klinisch-Chemisches Institut des Krankenhauses München-Schwabing
8 München 40, Kölner Platz 1, West Germany

Received 6 September 1975

1. Introduction

Much work has been spent in recent years on the mechanisms which are responsible for the regulation of the interconvertible pyruvate dehydrogenase (PDH) complex in mammalian tissues, in vivo. Concerning the various body organs, skeletal muscle has attained very little attention until now. In extension of our running interest in this field we have therefore studied at what proportion the active (dephospho) form (PDH_a) and the inactive (phospho) form (PDH_b) of the PDH complex do exist in skeletal muscle of rats under normal and special metabolic conditions such as diabetes, fasting and fasting-refeeding carbohydrate. Furthermore it seemed of interest to investigate the influence of muscular contraction on the steady state of the two molecular forms of the PDH system. A brief report on some of our results has been given elsewhere [1].

2. Materials and methods

Male Sprague–Dawley rats (130–160 g; Fa. Wiga, Sulzfeld, West-Germany) fed ad libitum with a standard laboratory chow (Labortierfutter 57 Z, J. Zahn, II Hockenheim, West Germany) were used. In the refeeding experiments the animals were given a high carbohydrate diet (Zwieback) after a 60 h fasting period. Diabetes was induced by s.c. injection of streptozotocin (100 mg/kg body weight) obtained by the Upjohn Company (Kalamazoo, Mich., USA). The diabetic rats were treated for 8–10 days with insulin (2–4 IU Altinsulin Hoechst s.c.), and used for the experiments 24–36 h after withdrawal of insulin.

The gastrocnemius muscle was electrically stimulated by needle electrodes inserted directly in the muscle. Isometric contractions were induced by square wave electrical pulses (S D 5 Stimulatan, Fa. Grass Medical Instruments Quincy. Mass. USA) with a duration of 20 msec. and a frequency of 10/sec. The voltage was 2 V. Muscle samples were obtained by freeze-clamping the gastrocnemius muscle with a Wollenberger clamp precooled in liquid nitrogen, and were then pulverized in the frozen state mechanically for 5 sec (Microdismembrator, Braun, Melsungen, West-Germany). A sample from the collateral non-stimulated hind limb served as control. The frozen muscle powder was homogenized with a five-fold amount (v/w) of ice-cold 20 mmol/l Tris buffer, pH 7.0, containing 2% lubrol, 4 times 30 sec. each, by a high speed tissue disintegrator (Ultraturrax 10 M, Janke and Kunkel, Stauffen i. Br., West Germany). Care was taken, that the temperature did not increase over 1°C during homogenisation. The homogenates were kept in liquid nitrogen until used for the PDH assay. After thawing, insoluble material was separated by centrifugation at 12.000 *g* for 1 min. PDH_a and total PDH activity were determined in the clear supernatant as described previously [2], ATP and creatine phosphate were estimated according to [3], ADP according to [4], and protein by the biuret method. All chemical reagents were products of E. Merck AG (Darmstadt, West Germany), and all biochemicals of Boehringer (Mannheim, West Germany). Lubrol 12 A 9 was a generous gift from the Deutsche ICI GmbH (Frankfurt, West Germany).

North-Holland Publishing Company – Amsterdam

Table 1
PDH-activities of rat gastrocnemius muscle at rest (non-stimulated hind leg), and after 10 min of isometric contraction (opposite hind leg)

	Rest			After stimulation		
	PDH_a	$PDH_{tot.}$	%	PDH_a	$PDH_{tot.}$	%
Normal fed	2.3 ± 0.2 (15)	14.7 ± 0.6 (15)	15.5 ± 0.9 (15)	5.6 ± 0.4[b] (14)	15.2 ± 0.7 (14)	40.8 ± 1.8 (14)
Diabetes	1.2 ± 0.1[a] (14)	13.0 ± 0.5 (14)	9.3 ± 0.9 (14)	21.0 ± 0.1[b] (10)	13.0 ± 0.4 (10)	16.7 ± 1.3 (10)
60h - fasted	0.78 ± 0.03[a] (21)	13.1 ± 0.4 (21)	6.4 ± 0.2 (21)	1.7 ± 0.2[b] (12)	14.6 ± 0.5 (12)	12.6 ± 1.4 (12)
60h - fasted 5h -refed	1.8 ± 0.1 (8)	11.9 ± 0.5 (8)	15.2 ± 0.5 (8)	4.7 ± 0.2[b] (8)	12.3 ± 0.4 (8)	39.0 ± 2.0 (8)

[a] Significant as compared to PDH of the normal fed rat ($P < 0.05$).
[b] Significant as compared to the corresponding value at rest ($P < 0.05$).
Enzyme activities are given in mU (nmol acetyl-CoA formed per min) × mg protein^{-1} ± S.E.M. Number of experiments in parantheses, for further experimental details see Materials and methods.

Statistical significance was determined by the Wilcoxon rank sum test as modified by Mann and Whitney [5].

3. Results and discussion

As indicated in table 1, 15.5% of total PDH activity is present in the active form in skeletal muscle of normal fed rats at rest. This level is markedly decreased in diabetic or fasted rats to 9.3% and 6.4% of total PDH activity, respectively. In all three groups total PDH activity remained essentially unchanged. On a wet weight basis total PDH activity in the resting skeletal muscle amounts to 1950 mU/g. If one sets the value of heart muscle (3340 mU/g) [6] to 100%, the relative total PDH activities of rat tissues are as follows: skeletal muscle 58, kidney 34, brain 28, liver 22, adipose tissue 3. This order of sequence changes on comparing the PDH_a activities relative to heart muscle PDH_a (2320 mU/g = 100%): kidney 35, brain 28, skeletal muscle 14, liver 5, adipose tissue 1.3 [2, 6–8].

The decrease in PDH_a activity of skeletal muscle due to fasting or diabetes in a phenomenon which has also been observed to occur in other tissues [2,6,8] with the exception of brain [7]. For an explanation it was suggested that it is the increased supply and oxidation of long chain fatty acids which favours the transition of PDH_a to PDH_b – possibly due to elevation of the mitochondrial ATP/ADP ratio [9,10]. If the mobilisation of free fatty acids is turned down by refeeding the fasted rats with a carbohydrate-rich diet, PDH_a activity returns back towards the normal range within several hours (table 1). This is in accordance with our previous observations in other tissues [2,6].

The percentage of PDH_a on total enzyme activity increased from 15.5 to 40% after electrical stimulation of the gastrocnemius muscle (table 1). An increase of PDH_a activity, though smaller, was seen also in the stimulated muscle of diabetic and fasted rats, resulting in 16.7% and 12.6% of the active form, respectively.

The effect of electrical stimulation is markedly enlarged if the gastrocnemius muscle is subjected to mechanical work. As shown in table 2 PDH_a arrives at 60% of total PDH activity under working conditions – the highest activity of the enzyme we could observe during the current investigations.

Fig.1 illustrates the time dependency of PDH activation during electrical stimulation. As may be seen there is a clear increase in enzyme activity already after 5 sec, nearly maximal activation being achieved after 1 min of stimulation. A similar relationship is obtained in the muscle from 60 h fasted rats, yet at a much lower level of PDH_a formation.

Table 2
PDH-activities of the working gastrocnemius muscle of normal fed rats

	PDH_a (mU/mg protein)	PDH_{total}	PDH_a percent of total
resting mucle	2.5 ± 0.2 (18)	16.9 ± 0.6 (18)	14.5 ± 0.9 (18)
working muscle	10.3 ± 0.5 (18)	17.0 ± 0.4 (18)	61.2 ± 2.7 (18)

A weight of 51 g moving on a roll was tied to one of the hind limbs, and the gastrocnemius muscle was electrically stimulated with a frequency of 1 pulse/ sec. at 8 V over a period of 5 min. The muscle of the other non-stimulated hind limb served as control. For further experimental details see under Materials and methods.

As illustrated in fig.2 the activation, i.e. dephosphorylation of PDH in contracting muscle is a readily reversible process. Immediately after electrical stimulation is discontinued, rephosphorylation sets in leading to a decrease of PDH_a to almost the original levels within a few minutes. Fig.2 furthermore shows that the time course of PDH activation and inactivation during stimulation and recovery, respectively, is closely matched by a decrease and a restoration of the phosphocreatine levels. As expected ATP and ADP showed essentially no changes. Thus, in skeletal muscle an interrelationship appears to exist between the phosphorylation state of the phosphocreatine/creatine system and that of the PDH system, quite similar to that between the phosphorylation state of the adenine nucleotides and PDH observed in liver [9,10] and fat cell mitochondria [11]. A direct interaction between phosphorycreatine and the PDH system can be excluded from studies on the purified heart muscle enzyme indicating that phosphocreatine cannot replace ATP as a substrate for the kinase reaction [12]. We should like to suggest therefore that there is a drop of the mitochondrial ratio of ATP/ADP during muscle contraction, and that this may trigger PDH_b to PDH_a conversion, similarly as discussed for other tissues [9–11]. Unfortunately no possibility exists to date for quantifying the mitochondrial levels of adenine nucleotides in

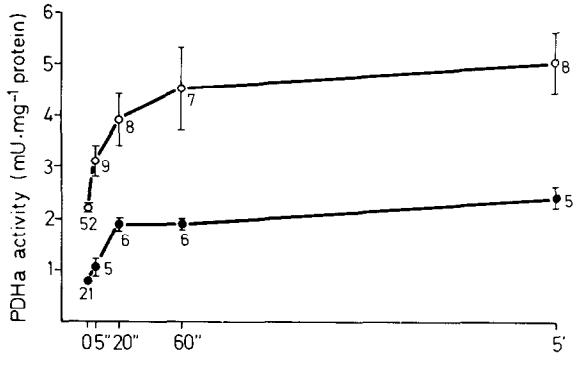

Fig.1. Time course of PDH_a formation during isometric muscle contraction. Experimental details see table 1. (○——○) Normal fed rats; (●——●) 60 h fasted rats. Numbers of experiments are indicated in parentheses, vertical bars represent S.E.M.

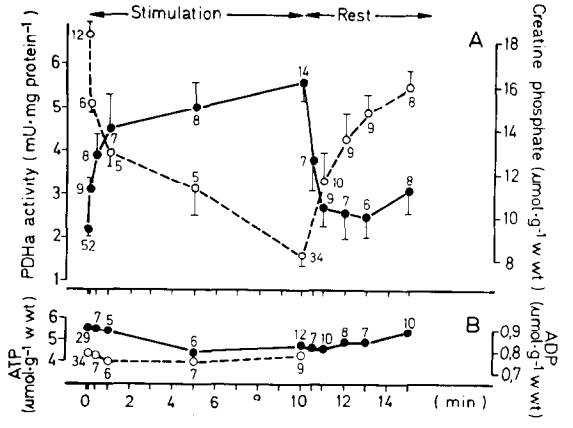

Fig.2. PDH_a, creatine phosphate, and adenine nucleotides of gastrocnemius muscle of normal fed rats during electrical stimulation and recovery. (A) (●——●) PDH_a; (○– – –○) creatine phosphate. (B) (●——●) ATP; (○– – –○) ADP. Numbers of experiments are given in parentheses, vertical bars represent S.E.M.

skeletal muscle. It seems, however, well possible that, due to the action of the mitochondrially bound creatine kinase present in skeletal muscle [13], the phosphocreatine/creatine system in the cytosol is linked to the mitochondrial adenine nucleotide system, and that a fall of the mitochondrial ATP/ADP is reflected by a decrease of the phosphocreatine level.

Acknowledgements

G. H. is a recipient of an Ausbildungsstipendium der Deutschen Forschungsgemeinschaft, Bad Godesberg, Germany, which also supported this work. We thank Miss E. Daller for her excellent technical assistance.

References

[1] Henning, G., Löffler, G. and Wieland, O. H. (1975) XIth Annual Meeting of the European Association of the Study of Diabetes, München, Abstract No. 108.
[2] Wieland, O. H., Patzelt, C. and Löffler, G. (1972) Eur. J. Biochem. 26, 426–433.
[3] Lamprecht, W. and Trautschold, J (1974) in: Methoden d. Enzymat. Analyse (Bergmeyer, H. U., ed.) 3rd Edn. Vol. 2 (Verlag Chemie, Weinheim/Bergstr.) pp. 2151–2160.
[4] Jaworek, D., Gruber, W. and Bergmeyer, H. U. (1974) in: Methoden d. Enzymat. Analyse (Bergmeyer, H. U., ed.) 3rd Edn. Vol. 2 (Verlag Chemie, Weinheim/Bergstr.) pp. 2178–2181.
[5] Sachs, L. (1972) Statistische Auswertungsmethoden 3rd Edn. pp. 244–246, Springer Verlag, Berlin, Heidelberg, New York.
[6] Wieland, O., Siess, E., Schulze-Wethmar, F. H., Funcke, H. G. v. and Winton, B. (1971) Arch. Biochem. Biophys. 143, 593–601.
[7] Siess, E., Wittmann, J. and Wieland, O. (1971) Hoppe-Seyler's Z. Physiol. Chem. 352, 447–452.
[8] Weiss, L., Löffler, G. and Wieland, O. H. (1974) Hoppe-Seyler's Z. Physiol. Chem. 355, 363–377.
[9] Wieland, O. H. and Portenhauser, R. (1974) Eur. J. Biochem. 45, 577–588.
[10] Siess, E. A. and Wieland, O. H. (1975) FEBS Lett. 52, 226–230.
[11] Wieland, O. H., Weiss, L., Löffler, G., Brunner, J. and Bard, S. (1973) in: Metabolic Interconversion of Enzymes (Fischer, E. H., Krebs, E. G., Neurath, H. and Stadtman, E. R. eds.) p. 117–129, Springer Verlag Berlin, Heidelberg, New York.
[12] Siess, E. A. personal communication.
[13] Jakobs, H., Heldt, H. W. and Klingenberg, M. (1964) Biochem. Biophys. Res. Comm. 16, 516–521.