Volume 238, number 1, 71–73 FEB 06326 September 1988

Contractile activity enhances the synthesis of hexokinase II in rat skeletal muscle

F.E. Weber and D. Pette

Fakultät für Biologie, Universität Konstanz, D-7750 Konstanz, FRG

Received 26 July 1988

An 11-fold increase in hexokinase activity and the hexokinase II isoform was found in rat tibialis anterior muscle after 7 days of chronic, low-frequency stimulation. In vivo labeling studies showed that this increase in enzyme protein content was related to an approx. 30-fold increase in [^{35}S]methionine incorporation.

Hexokinase; Enzyme activity; Hexokinase II protein; Protein labeling; Chronic stimulation; (Skeletal muscle)

1. INTRODUCTION

Hexokinase (HK, EC 2.7.1.1) is present in skeletal muscle in two isoforms, HKI and HKII, of which the latter is predominant [1]. The activity level of HK in muscle is regulated by insulin [2] and, additionally, by contractile activity. Increases in HK activity have been demonstrated in exercising muscle [3–5] and muscles subjected to chronic, low-frequency stimulation [6–8]. Electrophoretic [6] and immunochemical (Weber and Pette, unpublished) analyses indicate that chronic stimulation mainly induces an elevation in HKII. The present study was undertaken to investigate, in more detail, the time course of HK activity in chronically stimulated muscle and to examine to what extent it correlates with changes in the HKII protein and its synthesis.

2. MATERIALS AND METHODS

2.1. *Animals, stimulation, muscle extracts, HK activity*

Adult male Wistar rats were subjected to chronic stimulation of the left common popliteal nerve (10 Hz, 10 h/day) as described [7]. After various time periods, the animals were killed and tibialis anterior (TA) muscles of stimulated and contralateral legs were dissected and frozen in liquid N_2. Muscles were extracted and total HK activity was photometrically determined [7,9].

Correspondence address: D. Pette, Fakultät für Biologie, Universität Konstanz, Postfach 5560, D-7750 Konstanz, FRG

2.2. *Immunochemical quantification of hexokinase II*

Hexokinase II was purified by affinity chromatography from rat skeletal muscle according to [10]. Antibodies were raised in sheep and IgG fractions biotinylated according to [11]. Hexokinase II protein was visualized on one-dimensional electrophoreses [12] by immunoblotting [13] and quantified by a sandwich enzyme-linked immunosorbent assay (ELISA) [14].

2.3. *In vivo labeling and immunoprecipitation of HKII*

Rats were disconnected from the stimulator, anesthetized, and a mixture of 18 μl of [^{35}S]methionine (equivalent to 270 μCi, Amersham Buchler, Braunschweig) and 2 μl India ink was injected according to [15] into the midbelly of contralateral and stimulated TA muscles. After 2 h, rats were killed and the ink-marked muscle portions were excised and extracted for immunoprecipitation [16] of HKII. A shortcoming of the in vivo labeling method [15] is that large variations may exist between the amounts of [^{35}S]methionine applied in each experiment. To compare the radioactivities incorporated into HKII in contralateral and stimulated muscles, measurements were also performed on immunoprecipitated troponin-C and troponin-I, as well as on the electrophoretically separated α- and β-tropomyosin subunits. Experimental evidence (unpublished) indicates that chronic stimulation up to 7 days does not measurably alter the synthesis rates of these myofibrillar proteins. Radioactivities incorporated into these proteins were determined in both contralateral and stimulated TA muscles and used as reference values to calculate the amounts of immunoprecipitated HK protein which were applied for one-dimensional electrophoresis. The autoradiographs of the separating gels were densitometrically evaluated. Alternatively,

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

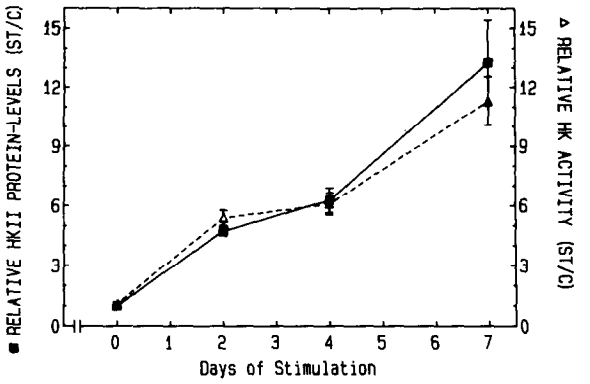

Fig.1. Increases in total hexokinase activity and the hexokinase II protein in chronically stimulated (10 Hz, 10 h/day) rat tibialis anterior muscle. Values (means ± SE) in the stimulated muscles have been referred to the values in the corresponding contralateral unstimulated muscles. Each time point represents independent measurements on 4 rats.

the HKII was extracted from the corresponding gel piece and its radioactivity determined.

3. RESULTS

Chronic stimulation induced rapid and pronounced increases in both HK activity and the immunochemically assessed amount of the HKII protein. A 5-fold increase in HKII occurred after only 2 days of stimulation and after 7 days, a 12-fold increase was found (fig.1). The similar time courses of the increases in total HK activity and HKII protein indicated that the rise in enzyme activity was a result of the increase in the HKII protein. In vivo labeling studies were performed to investigate whether the increase in enzyme protein was brought about by enhanced synthesis. The radioactivity incorporated into HKII was greatly increased after 7 days of stimulation (fig.2). Quantitative evaluations showed that [^{35}S]methionine incorporation into HKII increased by 30-fold after 7 days of stimulation (table 1).

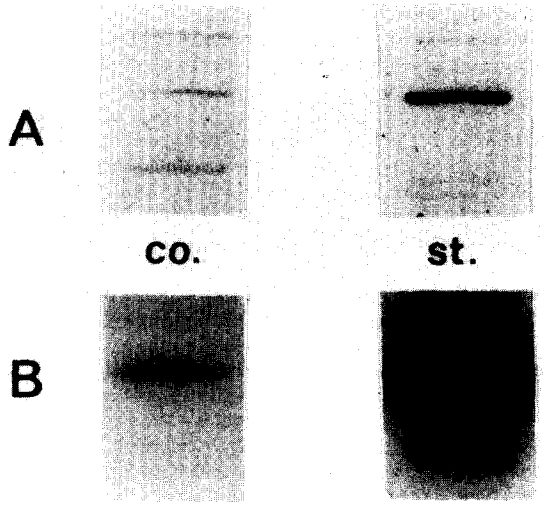

Fig.2. Effect of 7-day chronic stimulation on the protein amount of hexokinase II and [^{35}S]methionine incorporation into the enzyme. (A) Immunoblot of hexokinase II after one-dimensional electrophoresis. (B) Autoradiograph of [^{35}S]methionine-labeled hexokinase II after immunoprecipitation and one-dimensional electrophoresis. Co., contralateral; st., stimulated.

Table 1

Effect of chronic low-frequency stimulation on total hexokinase activity (U/g muscle) and [^{35}S]methionine incorporation (cpm)[a] into hexokinase II protein of rat tibialis anterior muscle (co., contralateral; st., stimulated)

	Enzyme activity			[^{35}S]Methionine incorporation		
	co.	st.	st./co.	co.	st.	st./co.
Rat 1	2.6	17.7	6.8	500	9607	19.2
Rat 2	2.5	26.9	10.8	650	25220	38.8
Rat 3	2.7	22.8	9.9	1183	28144	23.8
Rat 4	2.5	27.7	11.8	1295	36732	28.4
Means ± SE	9.8 ± 1.8			27.5 ± 7.2		

[a] Due to the variable application of [^{35}S]methionine to contralateral and stimulated muscles, radioactivity incorporated into hexokinase II was normalized according to the measured [^{35}S]methionine incorporation into tropomyosin, troponin-C and troponin-I. This evaluation allows a comparison between the data from contralateral and stimulated muscles in each animal

4. DISCUSSION

Our results support the idea that the pronounced elevation in HK activity induced in rat fast-twitch muscle by low-frequency stimulation results from an increase in HKII protein synthesis. The increase in HKII reaches its maximum after 1 week [7], i.e. at a time when the enzyme activities of aerobic-oxidative metabolism which are also augmented by chronic stimulation [7] have not yet attained their maximal values. The rapid increase in HK activity suggests that the stimulated muscle initially bases

its energy supply on glucose catabolism. This necessity of enhanced blood glucose utilization is supported by the previous finding that chronic stimulation leads to a rapid exhaustion of the muscular glycogen stores [17]. In addition, an increased rate of glucose uptake, in both the absence and presence of insulin, is induced by exercise in rat muscle [18]. The finding that HKII is the only enzyme of the glycolytic pathway which is elevated by sustained contractile activity indicates that glucose phosphorylation might also be an important step in the control of glucose utilization by skeletal muscle.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft, Sonderforschungsbereich 156. The authors thank K.-T. Härtner for the anti-troponin antibodies and Dr J.-A. Simoneau for helping with the in vivo labeling experiments.

REFERENCES

[1] Katzen, H.M. and Schimke, R.T. (1965) Proc. Natl. Acad. Sci. USA 54, 1218–1225.
[2] Katzen, H.M. (1967) Adv. Enzyme Regul. 5, 335–356.
[3] Peter, J.B., Jeffres, R.N. and Lamb, D.R. (1968) Science 160, 200–201.
[4] Barnard, R.J. and Peter, J.B. (1969) J. Appl. Physiol. 27, 691–695.
[5] Green, H.J., Reichmann, H. and Pette, D. (1983) Pflügers Arch. 399, 216–222.
[6] Pette, D., Smith, M.E., Staudte, H.W. and Vrbová, G. (1973) Pflügers Arch. 338, 257–272.
[7] Simoneau, J.-A. and Pette, D. (1988) Pflügers Arch. 412, 86–92.
[8] Reichmann, H., Hoppeler, H., Mathieu-Costello, O., Von Bergen, F. and Pette, D. (1985) Pflügers Arch. 404, 1–9.
[9] Esterby, J.S. and O'Brian, M.J. (1973) Eur. J. Biochem. 38, 201–211.
[10] Holroyde, M.J. and Trayer, I.P. (1976) FEBS Lett. 62, 215–219.
[11] Guesdon, J.-L., Ternynck, T. and Avrameas, S. (1979) J. Histochem. Cytochem. 27, 1131–1139.
[12] Laemmli, U.K. (1970) Nature 227, 680–685.
[13] Towbin, H., Staehelin, T. and Gordon, J. (1979) Proc. Natl. Acad. Sci. USA 76, 4350–4354.
[14] Leberer, E. and Pette, D. (1986) Biochem. J. 235, 67–73.
[15] Matsuda, R., Spector, D.H. and Strohman, R.C. (1983) Dev. Biol. 100, 478–488.
[16] Seedorf, U., Leberer, E., Kirschbaum, B.J. and Pette, D. (1986) Biochem. J. 239, 115–120.
[17] Maier, A. and Pette, D. (1987) Pflügers Arch. 408, 338–342.
[18] Ivy, J.L. and Holloszy, J.O. (1981) Am. J. Physiol. 241, C200–C203.