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Rapid up- and down-regulation of hexokinase II in rat skeletal muscle in response to altered contractile activity

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An up to 14-fold increase in total hexokinase activity induced by low-frequency stimulation in rat fast-twitch muscle was followed by a rapid decay in enzyme activity after cessation of stimulation. In vivo labeling revealed that these alterations were related to rapid changes in [^{35}S]methionine incorporation into hexokinase II. A recovery period of 15 h after cessation of stimulation was sufficient to normalize the approximately 30-fold elevated [^{35}S]methionine incorporation.

Contractile activity; Hexokinase II; Protein synthesis; Fast-twitch muscle; (Rat)

1. INTRODUCTION

The catalytic activity of a regulatory enzyme can be modified by: (i) allosteric effectors; (ii) interconversion of active and inactive forms by chemical modification; or (iii) changes in the cellular amount of the enzyme protein. Hexokinase II, the predominant isozyme in skeletal muscle [1], is an enzyme regulated by the latter mechanism. We have previously shown that several-fold increases in total hexokinase (HK, EC 2.7.1.1) activity can be induced in fast-twitch muscles in small mammals by increased contractile activity [2–4]. These increases in total activity correspond to elevations in the protein amount of hexokinase II (HKII) and are brought about by an approximately 30-fold increase in its synthesis rate [5,6]. The purpose of the present study was to investigate in more detail the time course of increases and decreases in HK activity in response to altered amounts of contractile activity as induced by low-frequency stimulation and recovery after cessation of stimulation. In addition, we were interested in the time course of changes in HKII synthesis related to these changes in enzyme protein amount.

2. MATERIALS AND METHODS

2.1. *Chronic low-frequency stimulation*

The experiments were performed on adult male Wistar rats. Chronic low-frequency stimulation (10 Hz, 10 h daily) was performed via electrodes implanted laterally to the peroneal nerve of the left hindlimb as previously described [4]. The animals were killed after various periods of stimulation. Stimulated and contralateral tibialis anterior muscles were excised, frozen in liquid N_2 and stored at −70°C.

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2.2. *Measurement of total HK activity and determination of HKII by a sandwich ELISA*

Frozen muscles were pulverized under liquid N_2 and extracted in a 19-fold volume of extracting medium (100 mM phosphate buffer, 2 mM EDTA, 1% Triton X100, pH 7.2). Total HK activity was measured photometrically at 30°C according to Easterby and O'Brien [7]. The immunochemical determination of HKII protein was carried out by means of the previously described isozyme-specific sandwich ELISA [5,8].

2.3. *In vivo labeling by [^{35}S]methionine incorporation*

The same methods were used as previously described [5,9]. In short, 2 h before the end of the experiment, the animal was anesthetized and experimental and contralateral tibialis anterior muscles were partially exposed in situ and a mixture of 18 μl of [^{35}S]methionine (equivalent to 270 μCi, Amersham Buchler, Braunschweig) and 2 μl India ink was injected into the midbelly of the muscles. The skin was closed by surgical clips and the animal was kept for 2 h under anesthesia. Thereafter, the animal was killed and the ink-marked muscle portions were excised and, after pulverization under liquid N_2, extracted for immunoprecipitation of HKII with a polyclonal sheep anti-rat HKII antibody [5]. The precipitated HKII protein was subjected to one-dimensional electrophoresis in the Laemmli system [10] and visualized by autoradiography. The autoradiographs were evaluated densitometrically. For comparison, we also measured [^{35}S]methionine incorporation into immunoprecipitated (troponin-C, troponin-I) or electrophoretically separated (α- and β-tropomyosin) reference proteins [5,9].

3. RESULTS

In agreement with previous results [4–6], chronic low-frequency stimulation induced a steep increase in total HK activity in rat fast-twitch muscle (fig.1). The increase in enzyme activity was maximal during the first 7 days of stimulation. Thereafter, the increase became less steep and the HK activity reached its maximum by 14 days [4]. Prolonged stimulation (>21 days, data not shown) was followed by a steep decrease in activity.

The steep slope of the initial increase prompted us to determine, in a series of short-term stimulated animals,

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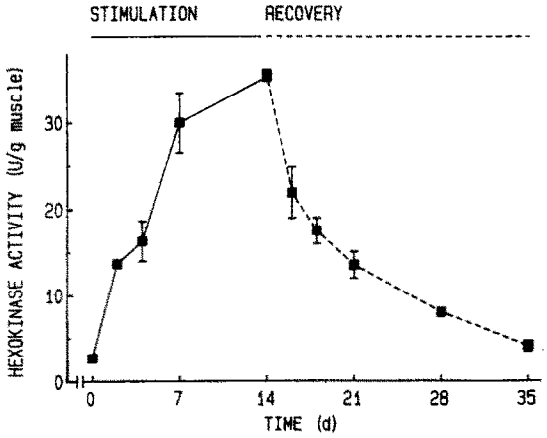

Fig.1. Changes in total HK activity in response to alterations in the amount of induced contractile activity of rat fast-twitch muscle. The first part of the graph (up to 14 days) illustrates the time course of the increase in enzyme activity in response to chronic low-frequency stimulation. The second part shows the decay in HK activity after cessation of stimulation on day 14. The values represent means ± SE for $n = 4$ animals for each time point.

the time point at which HK activity started to rise (fig.2). A 1.4-fold increase in total HK activity (significant on the basis of a paired two-tailed t-test) was observed as soon as 5 h after the onset of stimulation. After 1 day of stimulation, total HK activity was 2-fold elevated.

Rapid changes in total HK activity were not only observed with enhanced contractile activity by low-frequency stimulation, but occurred also in response to cessation of stimulation (fig.1). Disconnection of the animals from stimulation at the time point when HK had reached its maximum value (14 days), was followed by an abrupt decline in HK activity. The time course of the decay resembled a first order reaction with an apparent $t_{1/2}$ of maximally 2.8 days. After 2 days of recovery, the enzyme activity dropped to 60% of its maximal value, and after 28 days it reached a value 3-fold above normal.

In view of the rapid decay of HK activity after cessation of stimulation, we were interested in studying the time course of related changes in HKII synthesis. For this purpose, we used the in vivo labeling approach of Matsuda et al. [11] and measured [^{35}S]methionine incorporation into HKII. The enzyme protein was immunoprecipitated from muscle extracts of two groups of rats that had been stimulated for 6 days and 5 h. One group (a) was killed immediately after stimulation, the other (b) was allowed to recover and was killed 15 h after cessation of stimulation. A stimulation period of 6 days was chosen because this time point fell into the period of the steep rise in HK activity (fig.1). A 12-fold increase in HKII protein was found in groups a and b (fig.3). Obviously, cessation of stimulation for 15 h caused no effect on the protein amount of HKII. Conversely, pronounced differences existed between the two groups with regard to the incorporation of [^{35}S]methionine into HKII. The animals which were killed immediately after the stimulation period of 6 days and 5 h displayed an approximately 30-fold elevated incorporation as compared to the labeling of the enzyme in the contralateral unstimulated muscles. However, the 15-h recovery period in group b was sufficient to reduce the 30-fold elevated [^{35}S]methionine incorporation into HKII to normal values (fig.3).

4. DISCUSSION

The present results demonstrate that the cellular amount of HKII in rat skeletal muscle is subject to rapid alterations in response to changes in the amount of contractile activity. The stimulation-induced augmentation in HKII suggests that glucose phosphorylation becomes a limiting step in glucose

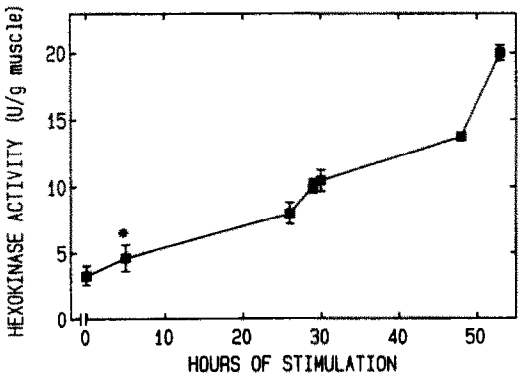

Fig.2. The initial phase of the increase in total HK activity of rat fast-twitch muscle as induced by low-frequency stimulation. The values represent means ± SE for $n = 4$ animals for each time point. Asterisk denotes significance ($P < 0.02$) of the increase after 5 h as determined in a two-tailed t-test.

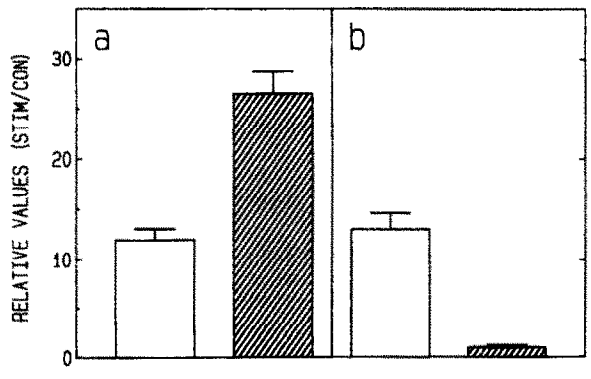

Fig.3. Immunochemically assessed protein amounts of HKII (open columns) and [^{35}S]methionine incorporation (hatched columns) into the enzyme protein of rat fast-twitch tibialis anterior muscle: (a) after 6 days and 5 h of low-frequency stimulation; (b) after 6 days and 5 h stimulation plus 15 h of recovery. The values (means ± SE, $n = 4$) of the stimulated (stim) muscles have been referred to the values of the unstimulated contralateral (con) muscles.

catabolism. Therefore, both the rapid rise in HK activity after the onset of low-frequency stimulation, as well as its decay after cessation of stimulation may be explained as adaptive changes in the glucose phosphorylating potential in response to altered demands of fuel supply. Obviously, these adaptive responses are due to rapid changes in the synthesis rate of HKII. As shown in the recovery experiment, a time period of only 15 h is sufficient to reduce the 30-fold elevated rate of synthesis to normal values. The decay in HKII following cessation of stimulation may be explained as resulting from a reduced rate of synthesis, although it cannot be excluded that enhanced degradation may also contribute. This suggestion is in agreement with previous results from our laboratory emphasizing the primary role of enzyme synthesis in determining the effective cellular levels of glycolytic enzymes in skeletal muscle [11,12].

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REFERENCES

[1] Katzen, H.M. and Schimke, R.T. (1965) Proc. Natl. Acad. Sci. USA 54, 1218–1225.
[2] Pette, D., Staudte, H.W. and Vrbová, G. (1972) Naturwissenschaften 8, 469–470.
[3] Pette, D., Smith, M.E., Staudte, H.W. and Vrbová, G. (1973) Pflügers Arch. 338, 257–272.
[4] Simoneau, J.-A. and Pette, D. (1988) Pflügers Arch. 412, 86–92.
[5] Weber, F.E. and Pette, D. (1988) FEBS Lett. 238, 71–73.
[6] Weber, F.E. and Pette, D. (1988) in: Sarcomeric and Non-Sarcomeric Muscles: Basic and Applied Research Prospects for the 90's (Carraro, U. ed.) pp.373–377, Unipress, Padova.
[7] Easterby, J.S. and O'Brien, M.J. (1973) Eur. J. Biochem. 38, 201–211.
[8] Leberer, E. and Pette, D. (1986) Eur. J. Biochem. 156, 489–496.
[9] Bär, A., Simoneau, J.-A. and Pette, D. (1989) Eur. J. Biochem. 178, 591–594.
[10] Laemmli, U.K. (1970) Nature 227, 680–685.
[11] Matsuda, R., Spector, D.H. and Strohman, R.C. (1983) Dev. Biol. 100, 478–488.
[12] Doelken, G. and Pette, D. (1974) Hoppe-Seyler's Z. Physiol. Chem. 355, 289–299.
[13] Illg, D. and Pette, D. (1979) Eur. J. Biochem. 97, 267–273.