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Electrostimulation-induced increases in fatty acid-binding protein and myoglobin in rat fast-twitch muscle and comparison with tissue levels in heart

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Chronic stimulation of rat fast-twitch muscle increased the content of both fatty acid-binding protein (FABP) and myoglobin. The increases in FABP, which reached values close to that of cardiac muscle, exceeded those in myoglobin and those in citrate synthase and 3-hydroxyacyl-CoA dehydrogenase activities.

Fatty acid-binding protein; Myoglobin; Aerobic-oxidative metabolism; Chronic electrostimulation; Contractile activity; (Rat fast-twitch muscle, Rat heart)

1. INTRODUCTION

Chronic low-frequency stimulation of fast-twitch muscle has been shown to induce pronounced increases in enzyme activities of aerobic-oxidative metabolism in a variety of small mammals [1]. In view of the increasing importance of the use of chronically stimulated skeletal muscle as cardiac substitute [2,3], we were interested in examining to what extent metabolic characteristics of low-frequency stimulated rat skeletal muscle resemble those of the cardiac muscle. Metabolic capacities of the muscle may not only be limited by enzyme activity levels, but also by substrate availability involving specific transport proteins. Therefore, we followed the time course of stimulation-induced changes in tissue levels of myoglobin and fatty acid-binding protein in rat tibialis anterior muscle and compared these results with those found in cardiac muscle. In addition, activity levels of reference enzymes of the citric acid cycle (citrate synthase) and fatty acid oxidation (3-hydroxyacyl-CoA dehydrogenase) were measured.

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2. MATERIALS AND METHODS

2.1. *Animals, chronic stimulation, muscles*

Adult male Wistar rats were used for chronic stimulation (10 Hz frequency, 10 h daily) of the left lateral peroneal nerve via implanted electrodes as described [1]. The animals were killed after various periods of stimulation, and stimulated and contralateral tibialis anterior muscles were excised, frozen in liquid N_2 and stored at −70°C. For comparison, slow-twitch soleus and heart muscles were also taken.

2.2. *Enzyme activities*

Frozen tissue samples were pulverized under liquid N_2, extracted, and measured for total citrate synthase (CS, EC 4.1.3.7) and 3-hydroxyacyl-CoA dehydrogenase (HADH, EC 1.1.1.35) activities as in [1,4]. Enzyme activities were expressed as U/g wet wt (means ± SE).

2.3. *Myoglobin and fatty acid-binding protein*

The muscle powder was suspended in 9 vols of 0.01 M acetic acid buffer (pH 4.5), extracted by sonication, and centrifuged for 15 min at 23000 × *g*. The supernatant fraction was used for electrophoresis of myoglobin and fatty acid-binding protein (FABP) in a 12–14% gradient polyacrylamide gel according to

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Ohno and Kuroshima [5]. For myoglobin determination, the Coomassie-blue-stained myoglobin band was cut out, and the dye eluting in 25% pyridine [6], was measured photometrically at 605 nm. Known amounts of purified myoglobin were used to establish a calibration curve. For determination of FABP, the proteins separated by one- [5] or two-dimensional (non-equilibrium pH gradient) electrophoresis (NEPHGE) [7] were transferred to a nitrocellulose membrane [8] and immunodecorated with a polyclonal rabbit-anti-rat heart FABP anitbody [9]. The antibody-antigen complex was visualized with the use of a peroxidase-coupled goat-anti-rabbit IgG antibody. The amounts of the reaction product were measured densitometrically taking into account the total area of the individual bands.

3. RESULTS

Immunoblot analyses of two-dimensional electrophoreses (NEPHGE) of soleus and stimulated tibialis anterior muscles with the anti-heart-FABP antibody identified one major and two minor proteins of similar mass (approx. 15 kDa) but slightly varying charges as FABP (fig.1A). The minor proteins of which one was more acidic and the other more alkaline were not separated from the major spot in either soleus or stimulated TA muscles. These minor spots probably represent charge variants of the FABP. Because the antibody used in this study showed some cross-reactivity with myoglobin (fig.1A), we preferred to quantitate FABP in muscle extracts by densitometrically evaluating immunoblots of one-dimensional gel electrophoreses (fig.1B). As is evident from the results plotted in fig.2, chronic stimulation of fast-twitch muscle induced a steep increase in FABP content reaching an approx. 3.5-fold higher level than in normal, unstimulated tibialis anterior between 21 and 28 days of stimulation. The attained level resembled that of soleus muscle and almost reached the cardiac muscle value which was 4.4-fold higher than that of unstimulated tibialis anterior (fig.2). The increase in HADH activity (from 10 ± 1 to 26 ± 1 U/g) was smaller than for FABP. Nevertheless, chronic stimulation raised the HADH activity of fast-twitch muscle to the level found in slow-twitch muscle (25 ± 1 U/g), but not to the level found in heart (84 ± 5 U/g) (fig.2).

The stimulation-induced increases in myoglobin content were less pronounced than for FABP (fig.3). Myoglobin increased 2.6-fold (from 1.7 ± 0.1 to 4.2 ± 0.1 mg/g) in 28 day stimulated tibialis anterior. However, it did not reach the values of soleus (5.6 ± 0.2 mg/g) or heart (6.0 ± 0.2 mg/g). Chronic low-frequency stimulation elicited similar increases (2.5-fold) in CS activity (from 18 ± 1 to

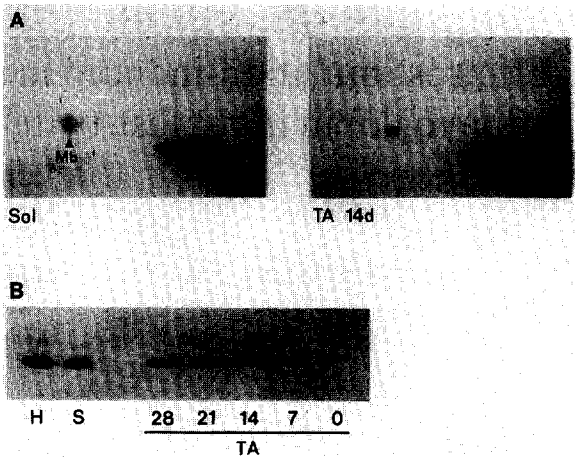

Fig.1. Immunoblot for fatty acid-binding protein (FABP) in normal and chronically stimulated rat muscle extracts. (A) Immunoblot after two-dimenisonal gel electrophoresis (NEPHGE) for soleus (Sol), and 14-day stimulated tibialis (TA) muscle of the rat. Mb, myoglobin. (B) Immunoblot after one-dimensional gel electrophoresis for heart (H), soleus (S) and chronically stimulated tibialis anterior muscle of the rat. Time of stimulation is given in days.

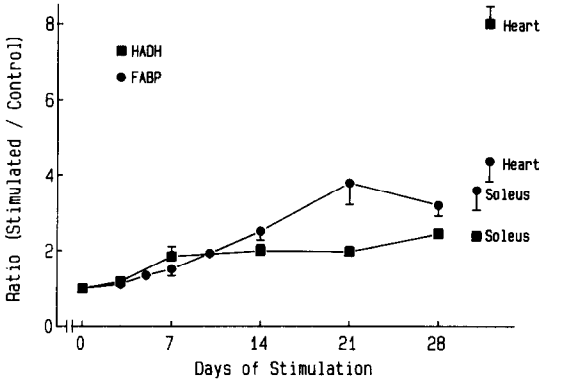

Fig.2. Time course of chronic low-frequency stimulation-induced increases in fatty acid-binding protein (FABP) and of 3-hydroxyacyl-CoA dehydrogenase (HADH) of rat tibialis anterior muscle. Values (means ± SE) in the stimulated muscles, as well as in heart and soleus muscles, have been referred to the values in control, unstimulated tibialis anterior muscle. Each time represents independent measurements on 4–5 rats.

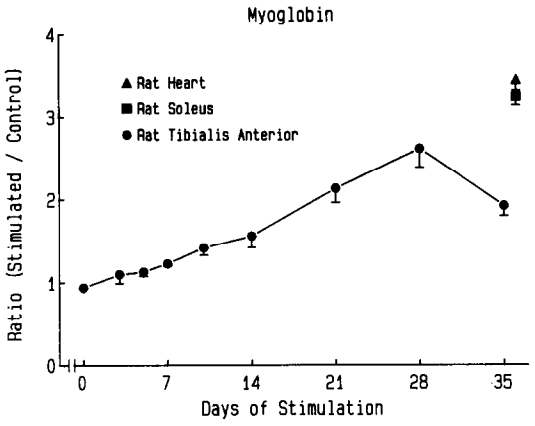

Fig.3. Time course of chronic low-frequency stimulation-induced increases in myoglobin of rat tibialis anterior muscle. Values (means ± SE) in the stimulated muscles, as well as in heart and soleus muscles, have been referred to the values in control, unstimulated tibialis anterior muscle. Each time represents independent measurements on 4–5 rats.

48 ± 3 U/g). The maximal level attained after 28 days stimulation exceeded that of soleus muscle (17 ± 1 U/g), but did not reach that found in heart muscle (91 ± 8 U/g).

4. DISCUSSION

It is known that pronounced differences exist between the tissue levels of FABP in different types of skeletal muscle [9–12]. These differences most likely reflect variable FABP contents in different muscle fiber populations. The present study demonstrates that increased contractile activity which previously has been shown to augment the aerobic-oxidative metabolic potential of the muscle [1], also induces a steep increase in the FABP content. This finding is of great interest with regard to the suggested role of FABP as an intracellular fatty acid transporter [10,11] and our recent observation of pronounced stimulation-induced increases in extracellular albumin content [13]. Albumin, the major extracellular fatty acid transporter, was approx. 6-fold elevated in chronically stimulated rabbit fast-twitch muscle and reached the level of soleus muscle [13]. The finding that chronic stimulation raised the FABP content almost to the level of cardiac muscle, could point to a limiting role of this transporter in fatty acid utilization during sustained contractile activity. This suggestion is corroborated by the finding that CS and HADH activities, used as indicators of terminal substrate oxidation, display much smaller increments in chronically stimulated muscle.

The observed increase in myoglobin content fits with the increase in oxygen consumption of chronically stimulated muscle [14] and also indicates that myoglobin may be a limiting factor of aerobic-oxidative metabolism in rat fast-twitch muscle. Its rise parallels that of CS, the selected mitochondrial reference enzyme of aerobic-oxidative metabolism. However, this close relationship may not exist in other species, particularly in human muscle, where induced increases in enzyme activities of aerobic-oxidative metabolism are not accompanied by increases in myoglobin content [15–17]. Myoglobin levels are high in human muscle and further increases may not be brought about by increased contractile activity. This is consistent with our notion that adaptive increases in skeletal muscle of components involved in the aerobic-oxidative metabolism are inversely related to their basal levels [1].

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