Succinate Dehydrogenase Activities of Fibers in the Rat Extensor Digitorum Longus, Soleus, and Cardiac Muscles

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Summary. Succinate dehydrogenase (SDH) activities and cross-sectional areas (CSAs) of different types of fibers in the superficial (EDLs) and deep (EDLd) regions of the extensor digitorum longus and soleus (SOL) muscles and the left ventricular muscle of the heart (HEART) of 10-week-old male rats were determined using quantitative histochemistry and a computer-assisted image processing system. The fibers were classified as type I, type IIA, type IIB, or type IIC according to their histochemically assessed adenosine triphosphatase activities. The mean SDH activity was higher and the mean CSA was smaller in type IIA fibers than in type IIB fibers in both the EDLs and EDLd. The mean SDH activity of type IIA fibers in the SOL was higher than that of type I fibers. Fibers in the HEART showed the highest mean SDH activity and the smallest mean CSA among all fiber types in the muscles examined. There was an inverse correlation between CSA and SDH activity for the different fiber types in different muscles. These data suggest that the SDH activity of fibers in muscle is fiber type- and size-specific, and that the highest SDH activity of fibers in the left ventricular muscle of the heart contributes to their functional properties, i.e., high fatigue resistance.

Mammalian skeletal muscles are composed of heterogeneous types of fibers based on their different myosin heavy chain (MHC) isoform expressions (Gunning and Hardeman, 1991; Pette and Staron, 1997). Muscle fiber types based on different MHC isoforms can be determined by immunohistochemical or enzyme histochemical techniques. The enzyme histochemical adenosine triphosphatase (ATPase) activities of muscle fibers based on their differential responses to alkaline and acid preincubations correspond to their MHC isoform profiles (Staron and Pette, 1986; Termin et al., 1989; Havenith et al., 1990). Based on differences in the ATPase activity following preincubations at different pHs, muscle fibers have been classified into two major types: acid-stable and alkali-stable type I (slow-twitch) and acid-labile and alkali-stable type II (fast-twitch). Type II fibers have been further subclassified into type IIA and type IIB fibers according to their ATPase activities in the acid range. Type IIA fibers become ATPase-negative earlier than type IIB fibers when the acidity of the preincubation medium is increased (Brooke and Kaiser, 1970a, b). Both type IIA and type IIB fibers contract rapidly, but type IIB fibers are more readily fatigued than type IIA ones. Type I fibers contract slowly and are less readily fatigued than type IIA and type IIB fibers. In addition, type IIC fibers, which are ATPase-positive irrespective of the pH of the preincubation medium, have been subclassified (Bottinelli et al., 1991; Hilber et al., 1997). Type IIC fibers are exclusively found at the embryonic and neonatal stages and decrease markedly during postnatal development (Smith et al., 1988, 1989; Wakata et al., 1990). The contractile property of a muscle depends on the proportions of the fiber types constituting it.

Skeletal muscles are composed of fibers having a wide range of mitochondrial enzyme activities even within the same fiber type (Nemeth and Pette, 1981; Pette, 1981; Sickle et al., 1982; Green et al., 1984; Reichmann, 1989). A quantitative histochemical technique was developed for measuring oxidative enzyme activity within individual muscle fibers (Pool et al., 1979a, b; Martin et al., 1985; Blanco et al., 1988). Previous studies (Reichmann and Pette, 1982; Punkt...
et al., 1989) demonstrated differences in the succinate dehydrogenase (SDH) activity of fibers of different types in different muscles or species. In this study, we examined the relationship between SDH activity and cross-sectional area of muscle fibers of different types, as defined by their enzyme histochemical ATPase activities, in the extensor digitorum longus and soleus muscles and the left ventricular muscle of the heart in rats. In addition, the intrafiber distribution of SDH activity was examined in a single muscle fiber. The SDH activities of muscle fibers correlate to their mitochondrial densities (Takekura and Yoshioka, 1987, 1990; Takekura et al., 1994) and functional properties, i.e., oxidative capacity (Edström and Kugelberg, 1968; Burke et al., 1971; Kugelberg, 1973; Kugelberg and Lindegren, 1979; Martin et al., 1988).

MATERIALS AND METHODS

Five ten-week-old Wistar male rats were used in this study. The mean body weight of the rats was 308.0 ± 11.1 g (n = 5). The right extensor digitorum longus (EDL) and soleus (SOL) muscles and the left ventricular muscle of the heart (HEART) were removed under sodium pentobarbital anesthesia (50 mg/kg body weight, i.p.). The SOL was chosen because this slow muscle is exclusively composed of high oxidative fibers over a cross-section, while the EDL was chosen because this fast muscle is composed of a mosaic pattern of fibers with both different sizes and oxidative enzyme activities. In addition, we separately examined the deep (EDLd) and superficial (EDLs) regions of the EDL because this muscle shows differing fiber type distributions between these two regions. The HEART was chosen because of the smaller sizes and higher oxidative enzyme activities of the muscle fibers.

The muscles were placed on cork at their in vitro length and quickly frozen in isopentane cooled with liquid nitrogen. Serial transverse sections, 10 µm thick, from the mid-belly of the muscle were cut on a cryostat at −20°C. Sections were brought to room temperature, air-dried for 30 min, and incubated for the demonstration of ATPase and SDH activities. For determination of the alkaline preincubation ATPase activity, the following procedures were employed: 1) preincubation for 15 min at room temperature in 75 mM glycine, 50 mM CaCl₂, and 75 mM NaCl in distilled water, adjusted to pH 10.4 with NaOH; 2) washing in 5 changes of distilled water; 3) incubation for 45 min at 37°C in 2.8 mM ATPase, 50 mM CaCl₂, and 75 mM NaCl in distilled water, adjusted to pH 9.4 with NaOH; 4) washing in 5 changes of distilled water; 5) immersion for 3 min in 1% CaCl₂; 6) washing in 5 changes of distilled water; 7) immersion for 3 min in 1% CoCl₂; 8) washing in 5 changes of distilled water; 9) immersion for 1 min in 1% (NH₄)₂S; 10) washing in 5 changes of distilled water; and 11) dehydration in graded ethanol with clearing in xylene and mounting. Basically, the same protocol was used for the acid preincubation conditions. However, acid preincubation was carried out for 5 min in 50 mM sodium acetate and 30 mM sodium barbital in distilled water, adjusted to pH 4.5 and 4.3 with HCl. Classification of fiber types was performed according to the staining intensities following the preincubation of barbital acetate buffers (pH 4.3 and 4.5) or glycine buffers (pH 10.4) (Brooke and Kaiser, 1970a, b). Thus, the classification into fiber types was based solely on the ATPase activity. The muscle fibers were classified as type I (positive at pH 4.3 and 4.5, and negative at pH 10.4), type IIA (positive at pH 10.4, and negative at pH 4.3 and 4.5), type IIB (positive at pH 4.5 and 10.4, and negative at pH 4.3), or type IIC (positive at pH 4.3, 4.5, and 10.4) (Fig. 1). The muscle fiber type distribution was calculated by counting the number of each type of fiber in the muscle. The distribution was determined from approximately 100 muscle fibers sampled from each region of the muscle.

The SDH activity was determined in an incubation medium containing 130 mM sodium succinate and 1.5 mM nitroblue tetrazolium in 0.1 M phosphate buffer, adjusted to pH 7.0. The reaction was stopped by multiple washings in distilled water and the sections were dehydrated in graded ethanol, passed through xylene, and then coverslipped. The SDH activities at 45 min of incubation time were used for comparison among fibers of different types in different muscles because the activities of the muscle fibers showed a plateau after 45 min of incubation.

The cross-sectional areas and SDH activities of fibers from each muscle or muscle region were examined using a computer-assisted image processing system (Neuromaging System) (Ishihara et al., 1995, 1997). Each pixel was quantified as one of 256 gray levels. A gray level value of zero was equivalent to 100% transmission of light, and that of 256 was equivalent to 0% transmission. The mean optical density (OD) value of all pixels within a fiber was determined using a calibration tablet which has 21 steps of gradient density ranges and corresponding diffused density values.
RESULTS

The fiber type distributions of each region in the EDL and of the SOL are summarized in Table 1. The superficial region of the EDL was composed of type IIA and type IIB fibers, while the deep region of the muscle was composed of type I, type IIA, and type IIB fibers. The SOL had type I and type IIA fibers as well as a few type IIC fibers. All fibers in the HEART could not be classified because they all showed positive staining by ATPase, irrespective of their preincubation pHs.

The mean CSA was greater and the mean SDH activity was lower in type IIB fibers than in type IIA fibers of the EDLs (Fig. 2). The mean CSA was greater and the mean SDH activity was lower in type IIB fibers than in either type I or type IIA fibers of the EDLd (Fig. 2). The mean SDH activity of type I fibers was lower than that of type IIA fibers in the SOL.

Fig. 1. Serial transverse sections of the superficial and deep regions of the extensor digitorum longus (EDL) and soleus (SOL) muscles and the left ventricular muscle of the heart (HEART) in a 10-week-old male rat. A-C: superficial region of the EDL. D-F: deep region of the EDL. G-I: SOL. J-L: HEART. A, D, G, and J: ATPase activity following preincubation pH at 10.4. B, E, H, and K: ATPase activity following preincubation pH at 4.5. C, F, I, and L: SDH activity. 1 type I, 2 type IIA, 3 type IIB, 4 type IIC. Scale bar in panel L indicates 100 μm for all panels.
Fibers in the HEART showed the smallest mean CSA and the highest mean SDH activity among all types of muscle fibers examined in this study (Fig. 2).

There was an inverse correlation between CSA and SDH activity for the different muscle fiber types examined in this study ($r = -0.933$, $n = 9$) (Fig. 3).

In type I and type IIA fibers of the EDLs and EDLd, the SDH activities were higher in the subsarcolemmal than in the intermyofibrillar region of a muscle fiber (Fig. 1). Similar results were observed in type IIA and type IIC fibers of the SOL.

**DISCUSSION**

The mammalian skeletal muscles represent a heterogeneous population of fibers, and these differences

<table>
<thead>
<tr>
<th></th>
<th>Type I</th>
<th>Type IIA</th>
<th>Type IIB</th>
<th>Type IIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDLs</td>
<td>11.2±4.1</td>
<td>88.8±4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EDLd</td>
<td>10.0±3.1</td>
<td>26.7±6.9</td>
<td>63.3±8.5</td>
<td></td>
</tr>
<tr>
<td>SOL</td>
<td>71.8±7.2</td>
<td>24.4±5.5</td>
<td></td>
<td>3.7±2.4</td>
</tr>
</tbody>
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EDLs and EDLd: superficial and deep regions of the extensor digitorum longus muscle, respectively, SOL: soleus muscle.

**Fig. 2.** Mean cross-sectional areas and succinate dehydrogenase activities of different types of fibers in the superficial (EDLs) and deep (EDLd) regions of the extensor digitorum longus and soleus (SOL) muscles and the left ventricular muscle of the heart (HEART) in rats. Means ± standard deviations ($n=5$). SDH: succinate dehydrogenase, OD: optical density. *$p<0.05$ compared with the value of type IIA in the EDLs, *$p<0.05$ compared with the value of both type I and type IIA in the EDLd, *$p<0.05$ compared with the value of type IIA in the SOL, *$p<0.05$ compared with the value of all types in the EDLs, EDLd, and SOL.
have been utilized to classify the muscle fibers into various groups or types which share similar properties (ARIANO et al., 1973; ARMSTRONG and PHELPS, 1984). The classification of muscle fibers on the basis of their SDH activities is usually performed by dividing the muscle fibers into two groups with low- or high-oxidative enzyme activity (PETER et al., 1972). However, most skeletal muscles exhibit a mosaic pattern of staining intensities in their muscle fibers after the histochemical reaction for SDH, and variations in the SDH activity of muscle fibers are considerable even within the same fiber type (POOL et al., 1979a, b; NEMETH and PETTE, 1981; PETTE, 1981; REICHMANN and PETTE, 1982; SICKLES et al., 1982; GREEN et al., 1984; REICHMANN, 1989). This study was undertaken in order to determine the activity of a mitochondrial marker enzyme, SDH, in single muscle fibers defined by their histochemical ATPase activities. Methods for the quantification of enzyme activities expressed by different gray levels have been developed using a microphotometric technique which allows quantitative histochemical fiber analyses in muscle (MARTIN et al., 1985; BLANCO et al., 1988).

The SDH activity of fibers was demonstrated by tetrazolium salt techniques in tissues in which activity is distributed either heterogeneously (skeletal muscle) or homogeneously (cardiac muscle). A previous study (PUNKT et al., 1989) observed that SDH activity is higher in fibers of the rat SOL than in those of the EDL. In addition, fibers in the rat HEART showed higher SDH activity than those in the EDL and SOL. However, this study (PUNKT et al., 1989) did not examine the fiber sizes or SDH activities of type-defined fibers in the muscle.

Type I fibers show the highest oxidative enzyme activity in humans (ESSEN et al., 1975; OLD and JOHNSON, 1989). This is not the case for rats. In fact, there was no difference in the mean SDH activity of fibers between type I and type IIA fibers in the EDL in this study. In addition, type IIA fibers showed higher mean SDH activity than type I fibers in the SOL, irrespective of their similar fiber sizes. It is suggested that the SDH activity of fibers in muscle is fiber type-specific.

The mammalian cardiac muscle has three distinct MHC isoforms: V1, V2, and V3 (SUGIURA and YAMASHITA, 1998), indicating that fibers in the muscle can be classified into several types including hybrid types, as shown in a previous study using rabbits (SEIDEN et al., 1989). We were unable to classify the muscle fibers into several types by histochemical ATPase staining because all fibers in the HEART showed positive staining by ATPase, irrespective of their preincubation pHs. This result is in agreement with a previous study (WEISSBERG et al., 1982) where all muscle fibers in rat ventricles had uniform staining activity using ATPase following preincubation at pH 10.5. In addition, no variation in size or SDH activity of fibers in the HEART was observed in this study. Thus, fibers in the HEART showed the smallest mean CSA and the highest mean SDH activity among all fiber types in the muscles examined. It is suggested that the high SDH activity of fibers in the cardiac muscle contributes to their functional properties, i.e., high fatigue resistance.

It is of interest that there was an inverse correlation between CSA and SDH activity for the different fiber types among the various muscles examined in this study. It is suggested that the SDH activity of fibers in muscle is fiber size-specific.

In type I, type IIA, and type IIC fibers in the EDL and SOL, SDH activities were higher in the subsarcolemmal than in the intermyofibrillar region in a given muscle fiber (Fig. 1). Previous studies (REICHMANN and WILDENAUER, 1991; BELL et al., 1992; MARTIN and EDDINGTON, 1992) have already found higher SDH activities in the subsarcolemmal region of a muscle fiber of rat SOL and cat tibialis anterior muscles. These findings coincide nicely when it is considered that mitochondria are highly dependent

![Fig. 3. The relationship between the cross-sectional area and succinate dehydrogenase activity of different types of fibers in the superficial (EDLs) and deep (EDLd) regions of the extensor digitorum longus and soleus (SOL) muscles and the left ventricular muscle of the heart (HEART) in rats. Each dot shows mean ± standard deviation from five animals. SDH: succinate dehydrogenase, OD: optical density. r = −0.933 (n = 9).](image-url)
on oxygen and substrates which are supplied to the muscle fiber from capillaries which are located close to the sarcolemmal membrane.

In conclusion, this study confirms that the SDH activity of muscle fibers is fiber type- and size-specific, and that SDH activity is relatively higher in the subsarcolemmal region of a high oxidative muscle fiber.

REFERENCES


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