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A Comparative Microphotometric Study of Succinate Dehydrogenase Activity Levels in Type I, IIA and IIB Fibres of Mammalian and Human Muscles

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Summary. Activity levels of succinate dehydrogenase (SDH) were determined kinetically by means of comparative microphotometric measurements in situ. Activities were correlated with fibre types classified histochemically according to Brooke and Kaiser (1970). Analyses of tibialis anterior muscles in the mouse, rat, guinea pig, rabbit, cat and the human showed pronounced variations in the activity profiles of type I, type IIA and IIB fibres of these muscles. Large scattering of enzyme activity existed in the three fibre populations. Overlaps of varying extent were found for the SDH profiles between the different muscles. Type I fibres reveal species differences in aerobic oxidative capacity. Whereas the majority of the IIB fibres in rabbit muscle tended to be low in SDH activity, the main fraction of this fibre population was characterized by high activities in mouse muscle. Similarly, the IIA fibre populations revealed opposite properties in mouse and rabbit muscles. These extremes as well as intermediate activity patterns indicate that no general scheme exists according to which the histochemically assessable myosin ATPase is correlated with the aerobic oxidative capacity of muscle fibres in various mammalian muscles.

Introduction

Quantitative analyses of metabolic enzyme activities in microdissected single muscle fibres (Spamer and Pette 1977, 1979, 1980; C. Lowry et al. 1978; Hintz et al. 1980; O. Lowry et al. 1980; Nemeth et al. 1981) as well as microphotometric measurements of histochemical enzyme reactions (Spurway 1980, 1981; Nemeth and Pette 1981 b) have indicated the existence of various muscle fibre populations with widely scattering metabolic profiles. These results raise the question to which extent correspondences exist between the two commonly used systems of histochemical muscle fibre classification. Peter et al. (1972) based fibre typing staining reactions for metabolic enzymes and for myofibrillar actomyosin ATPase. These authors distinguished fast-glycolytic (FG), fast-oxidative-glycolytic (FOG) and slow-oxidative (SO) fibres. The distinction of type I,

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IIA and IIB fibres by Brooke and Kaiser (1970) was solely derived from differences in the pH lability of myofibrillar actomyosin ATPase activity. The correspondence of these two classifications was questioned by previous qualitative studies on rat muscles (Nemeth et al. 1979; Nemeth and Pette 1980, 1981a). Although type I fibres were found to correspond to the SO type, and the majority of type IIA fibres fell in the FOG group, no correspondence existed between IIB and FG fibres. A large fraction of the IIB fibres revealed enzymatic properties characteristic of FOG fibres. These results were confirmed in a quantitative study in which succinate dehydrogenase activity was determined microphotometrically in type I, IIA and IIB fibres of rat hind limb muscles (Nemeth and Pette 1981b).

The objective of the present investigation was to extend these findings and to study aerobic oxidative capacities of type I, IIA and IIB fibres in tibialis anterior muscles of several mammalians and man. This comparative study investigates to which extent metabolic properties of these fibre types are similar in different species. Moreover, it intends to prove in a larger scope how far the two classification systems of Brooke and Kaiser (1970) and of Peter et al. (1972) can be considered to be compatible.

Materials and Methods

Tibialis anterior (TA) muscles were obtained from adult animals (C 57 BL/6 Kon mice, Kisslegg: Sprague-Dawley/SIV 50 rats, Kisslegg: Bfa/ZH (bunt) guinea pigs, Kisslegg: White New Zealand rabbits, European house cat). Human TA was obtained intra operationem from a middle aged female patient who was not immobilized before the operation. Human vastus lateralis muscle (VL) was obtained from an 18 year old male patient who was operated after fracture of the femur.

Whole muscles or fibre bundles were kept in a slightly stretched position and were frozen in melting isopentane (−160° C). Serial 8 µm cross-sections were cut at −25° C on a cryostat microtome. Myofibrillar actomyosin ATPase (myosin ATPase) was stained after preincubation at room temperature at pH 4.5–4.6 for distinction of type I, IIA and IIB fibres as modified from the original method of Brooke and Kaiser (1970). The following conditions were found to be optimal with regard to pH and duration of preincubation: mouse 10 min at pH 4.6, rat 8–12 min at pH 4.6, guinea pig 8 min at pH 4.6, rabbit 6 min at pH 4.6, cat 10 min at pH 4.5 and human 10–12 min at pH 4.6. Preincubation buffer consisted of 100 mM KCl in 100 mM sodium acetate adjusted to the appropriate pH under the glass electrode with 100% acetic acid. After preincubation sections were rinsed 30 s in distilled water and were then incubated 30 min at 37° C in the following reaction medium: 3 mM ATP, 30 mM $CaCl_2$, and 55 mM NaCl in 50 mM glycine/NaOH buffer adjusted to pH 9.4. Sections were washed 1 min in distilled water and were then incubated 3 min in a 1% $CaCl_2$ solution. Thereafter, sections were rinsed 1 min in distilled water and were incubated 3 min in a 2% $CoCl_2$ solution. After three successive washings in distilled water, sections were incubated 50 sec in 1% $(NH_4)_2S$. After rinsing with distilled water, sections were dehydrated in ethanol and mounted in Entellan (E. Merck). Solutions for preincubation and for incubation in the presence of ATP were made up freshly.

The reaction medium for succinate dehydrogenase (SDH) contained 100 mM phosphate buffer (pH 7.6), 5 mM ethylene-diaminotetraacetic acid (EDTA), 1 mM KCN, 0.2 mM phenazine methosulfate, 50 mM succinate, 1.5 mM nitro-blue tetrazolium. The medium was prepared freshly twice a day and was stored at room temperature in the dark.

Microphotometry

Microphotometric SDH activity determinations were made with a computer controlled Leitz MPV 2 microscope photometer (Pette et al. 1979; Pette and Wimmer 1979; Pette 1981) using the Leitz KINETIC program as software. This program is a modified and extended version of the original

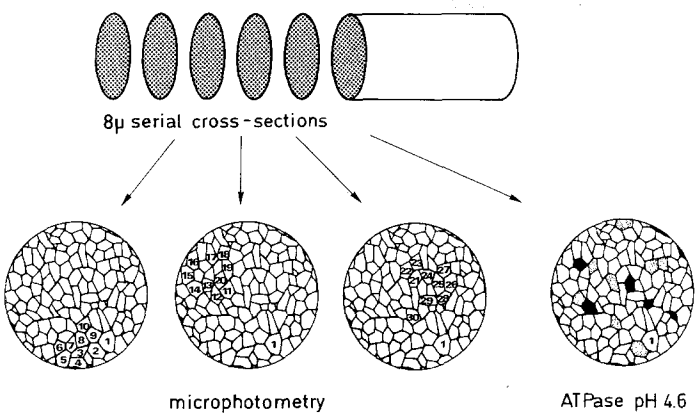

Fig. 1. Schematic representation of the procedure for comparative microphotometric enzyme activity determination in serial cross-sections of myosin ATPase typed muscle fibres. Enzyme activities determined in several fibres of one cross-section are referred to the activity of a reference fibre (no. 1) which is the same in all cross-sections

Leitz MPVIMP/LINREG program (Pette et al. 1979). It controls the complete measuring procedure with repetitive measuring cycles of variable duration at up to 24 randomly selected positions and contains subprograms for data processing. Measurements of extinction changes at 548 nm were taken in the fibre core using a round measuring diaphragm of 16 μm diameter in combination with a 25× objective. A freshly cut 8 μm thick section was mounted on a microscope slide. The slide was placed on a Leitz scanning stage (2.5 μm step width, 20 × 20 mm range) and up to 24 measuring positions were selected and stored. Two positions were usually chosen outside of the section in order to record nonspecific reaction of the medium. The measuring fields were placed exactly in the fibre cores avoiding the subsarcolemmal region. After setting the instrument to the appropriate number of measuring cycles, the reaction was started by pipetting 50 μl of the SDH medium on the section. A coverslip was quickly placed on the section. A U-shaped stainless steel wire was used as a spacer between glass slide and coverslip. Automatic recording of initial reaction velocities at the selected positions began 5–10 s after reaction onset. During 10 measuring cycles ten successive measurements were taken at each position. The time of a single measurement was 0.2 s. Total measuring time consisting of times for movement between the 24 positions and measurement during 10 cycles amounted to 60–90 s. Measurements were performed at room temperature. Data computing for maximum initial reaction rates were made by regression analysis. Nonspecific reactions recorded outside the tissue section were automatically subtracted. Correction for nonspecific reaction within the fibres was made by subtracting an average reaction rate determined in the absence of succinate (see Results).

Evaluation of relative SDH activities was made by referring the corrected absolute activities ($\Delta E_{548} \times s^{-1}$) measured in the various fibres of a section to that of a reference fibre. The latter was chosen according to shape and location so that it was easily detectable in serial sections. By referring all measurements to the same reference fibre in serial sections, relative SDH activity could be determined in a great number of fibres. The classification of the fibres as type I, IIA or IIB was made on parallel sections stained for myofibrillar actomyosin ATPase. The procedure of measurement for SDH activity in myosin ATPase typed fibres of serial cross-sections is illustrated schematically in Fig. 1.

Results

Control Reactions Without Substrate

With regard to nonspecific tetrazolium reduction, e.g. by "nothing dehydrogenase" (Ogawa and Shinonaga 1961), measurements were performed in the ab-

Table 1. Microphotometrically determined blank reaction rates (nothing dehydrogenase) in the absence of succinate in 8 μm cross-sectioned fibres of tibialis anterior muscles of various mammalians and the human

Species	Number of fibres	Reaction rate ($\Delta E_{548} \times 10^2 \times s^{-1}$) ($\bar{x} \pm SD$)	Coefficient of variation (%)
Mouse	37	1.149 ± 0.13	11
Rat	54	1.735 ± 0.35	20
Guinea pig	71	1.525 ± 0.29	19
Rabbit	70	1.601 ± 0.32	20
Cat	88	1.375 ± 0.31	23
Human	30	1.162 ± 0.13	11

sence of succinate. The recorded reaction rates were low and showed no fibre specific distributions. Blank reactions were determined in a great number of randomly selected fibres of each of the muscles investigated. As shown in Table 1, these measurements gave very low and similar reaction rates in the different muscles. Scattering of the blank reactions was in a range between 11% and 23% as judged from the coefficient of variations. In view of the low scattering and the low absolute values of the blank reaction, it appeared reasonable to correct the reaction rates determined in the presence of succinate for "nothing dehydrogenase" by subtracting an average blank reaction rate from each muscle. The experimental error of this procedure is negligible and is not greater than that arising from subtracting individual blank reactions determined for each fibre in parallel sections.

Reproducibility

An essential prerequisite for repetitive measurements at various positions is the reproducibility of the movements of the scanning stage to the selected positions in several measuring cycles. This was tested in several experiments in which identical positions within the same section were stored separately for independent measurements. A 100% reproducibility should give identical reaction rates for these measurements. Figure 2 illustrates an example of this type of experiment in which reaction rates for SDH were recorded in two independent measuring cycles in three different fibres. The graphical presentation of the reaction curves shows identity of the two independent measurements for each fibre (Fig. 2).

Determination of enzyme activity in fibres of serial sections raises the question to which extent variations exist in the reaction rates measured at different levels of the same fibre. In a previous investigation, measurements of SDH activity were performed along rat muscle fibres in longitudinal sections. These measurements gave a scattering in the range of $\pm 4.6\%$ suggesting an even distribution of enzyme activity (Pette et al. 1980). Assuming a uniform activity distribution along the fibres, measurements of relative activities in the same fibres of serial sections should provide information on the reliability of the method and its experimental error. Measurements of this type were performed in several experiments and gave identical results. Table 2 summarizes data of a typical experiment in which SDH activity was determined in 10 fibres in up to 5 serial sections. Differences in absolute activities due to uncontrolled

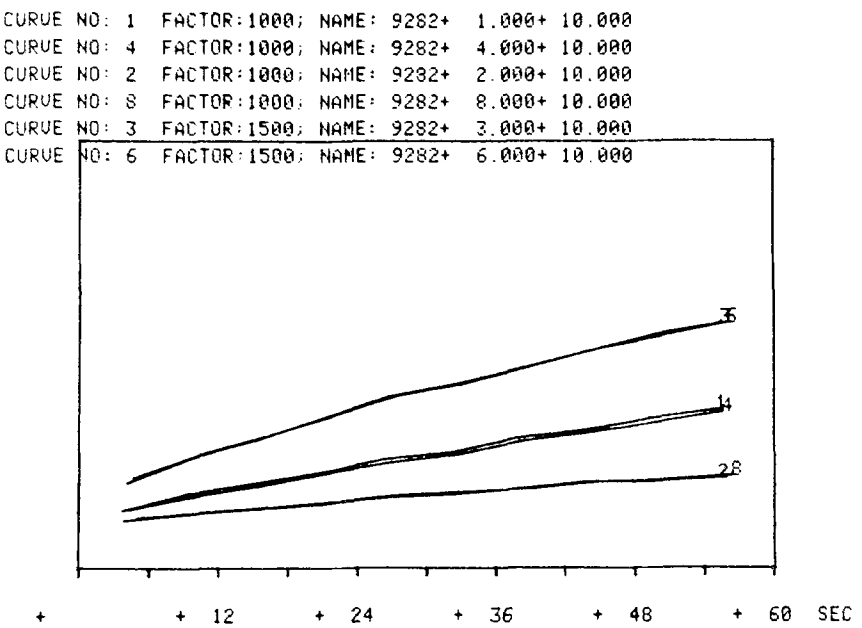

Fig. 2. Microphotometrically determined changes in absorbancy (548 nm) over time in 3 fibres of cross-sectioned (8 μm) rat TA muscle incubated for succinate dehydrogenase. In order to prove the reproducibility of the method, two independent enzyme activity determinations were performed for each fibre in separate measuring cycles, as illustrated by curves 1 and 4, curves 2 and 8 and curves 3 and 6. The figure is a photograph taken from the monitor's display

Table 2. Reproducibility of microphotometric succinate dehydrogenase activity measurements in 10 individual fibres of 5 serial cross-sections of rabbit tibialis anterior muscle

Fibre	Section 1	Section 2	Section 3	Section 4	Section 5	$\bar{x} \pm$ SD	Coefficient of variation [%]
1	0.72	0.67	0.65	0.80	0.57	0.682 ± 0.085	12.5
2	0.56	0.58	0.56	0.65	0.53	0.576 ± 0.045	7.8
3	0.64	0.60	0.66	–	–	0.633 ± 0.031	4.9
4	1.92	2.33	1.92	2.16	2.35	2.136 ± 0.211	9.9
5	0.61	0.55	0.55	0.48	0.61	0.56 ± 0.054	9.6
6	0.38	0.43	0.44	0.49	–	0.435 ± 0.045	10.3
7	0.52	0.56	0.41	–	0.51	0.5 ± 0.064	12.8
8	0.51	–	–	0.61	0.59	0.57 ± 0.053	9.3
9	1.54	–	1.41	1.40	1.55	1.475 ± 0.081	5.5
10	1.11	1.29	–	1.14	1.24	1.195 ± 0.084	7.0

variations in section thickness or in temperature, are eliminated by evaluation for relative activities (Pette et al. 1979; Pette and Wimmer 1980; Pette 1981). Scattering of the data in Table 2 may therefore be regarded as true experimental error. As is evident from these and other results (not shown), a mean coefficient of variation in the range of 9% has to be taken into account.

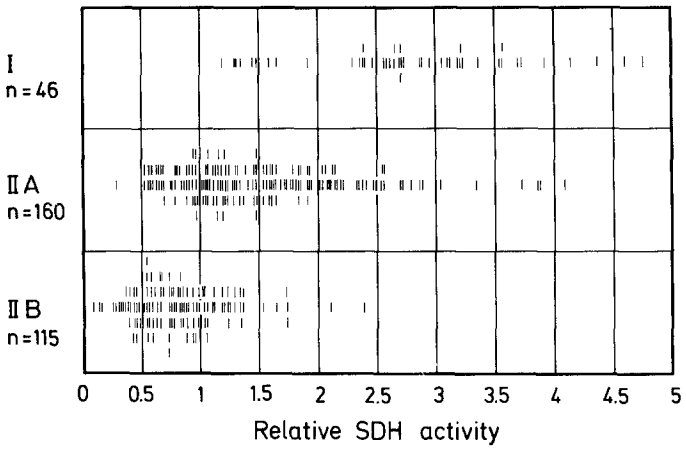

Fig. 3. Relative SDH activities in single type I, IIA and IIB fibres of cat TA muscle

SDH Activity in Typed Fibres

Measurement of SDH activity in fibres of serial cross-sections was done in the following way. A section was stained for SDH and several microphotographs were taken with a Polaroid camera accessory of the microscope photometer. A photomontage of the stained section was mounted from these micrographs. A low staining fibre of characteristic shape and topography, e.g. in the vicinity of a blood vessel or nerve, was chosen as reference fibre. The activity of this fibre was determined in each section and activities of other fibres were referred to it. The fibres selected for measurement were moved by remote control of the scanning stage into the measuring beam and their coordinates were stored by the computer. Their identity was marked in the photomontage by labelling them with numbers. This procedure facilitated the orientation and proved to be useful for selecting morphologically intact fibres. Furthermore, this method made it possible to perform a great number of measurements in serial sections and to relate the activity of each individual fibre to its myosin ATPase type which was established in a separate section and documented correspondingly by a photomontage. For evaluating all measurements taken on serial sections of the same block, the activity of each fibre was then corrected for the average blank reaction and was plotted according to its fibre type on a scale of relative SDH activity as illustrated in Fig. 3.

The scattering of microphotometrically assessed SDH activities in the three fibre populations is more pronounced than would be expected by visual examination of sections stained for SDH. Figure 4 gives two examples of qualitative SDH histochemistry with myosin ATPase stainings in parallel sections of cat (Fig. 4a) and human (Fig. 4b) TA muscle. As is seen in Fig. 3, type I fibres in TA of the cat vary about 4-fold between their extremes whereas an 8- to 10-fold variation in relative activities exists for the IIA and IIB fibres. Similar activity ranges were detected for human fibres (see Figs. 8 and 9): type I 5-fold, IIA 8-fold and IIB 10-fold.

The enzyme activity distribution in the three fibre populations of TA muscles

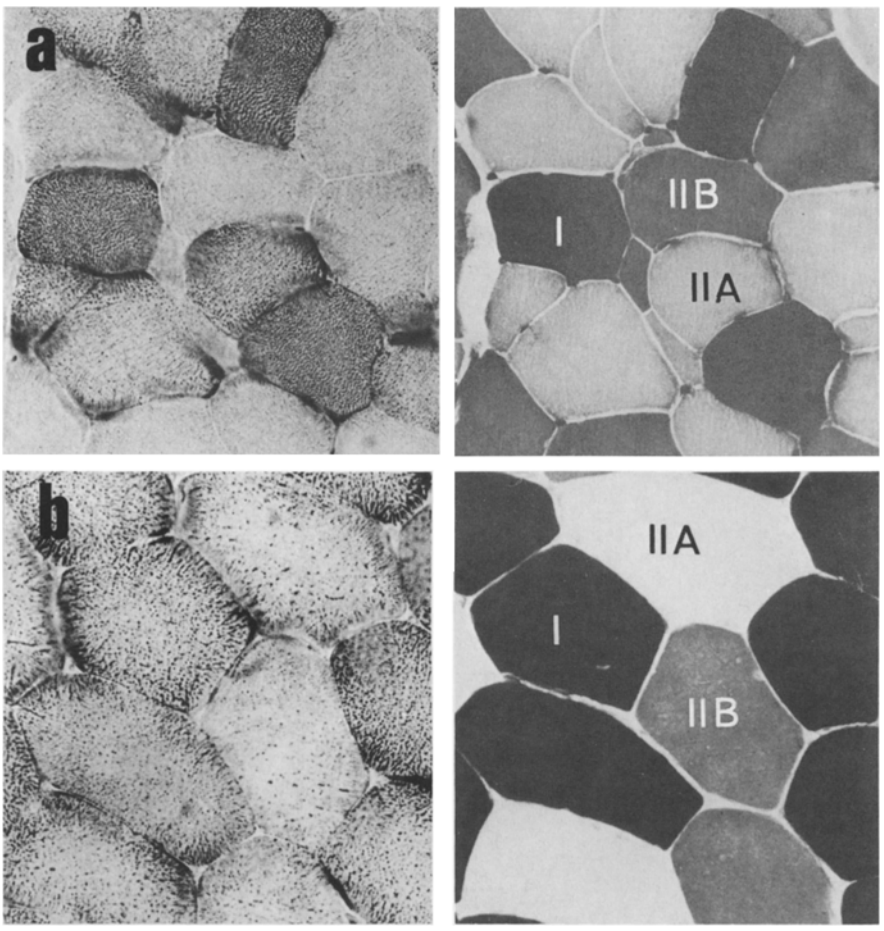

Fig. 4a, b. Staining for SDH (left) and for myofibrillar actomyosin ATPase after preincubation at pH 4.5 (cat) or 4.6 (human) in parallel sections of tibialis anterior muscles of the cat (**a**) and of the human (**b**). ×240

in various mammalians and man is best illustrated by the histograms depicted in Figs. 5–10 and Fig. 12. In some of the investigated TA muscles (mouse, guinea pig, rat and rabbit), type I fibres represented only a negligible fraction of the total fibre population (Ariano et al. 1973). Due to the small number, these data were omitted in the respective histograms.

In agreement with previous measurements on rat extensor digitorum longus and extensor hallucis longus muscles (Nemeth and Pette 1981b), IIA and IIB fibre populations of rat TA (Fig. 5) both show a wide spectrum of activities with a large overlap. The majority of the IIA fibres is in the higher activity range. About 95% of this population has relative activities higher than 1.5. A major fraction of the IIB fibres has a relative SDH activity between 1 and 1.5. However, 55% of the IIB's are found in the range of higher activities.

A similar activity distribution is seen for IIA and IIB fibres in TA muscles

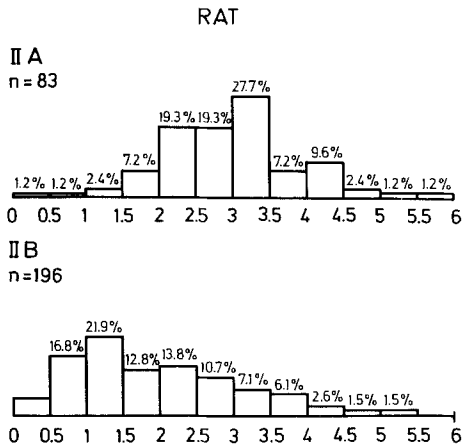

Fig. 5. Histograms of relative SDH activities in type IIA and IIB fibres of rat TA muscle

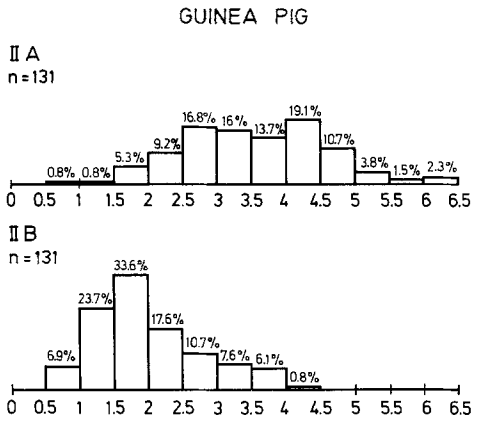

Fig. 6. Histograms of relative SDH activities in type IIA and IIB fibres of guinea pig TA muscle

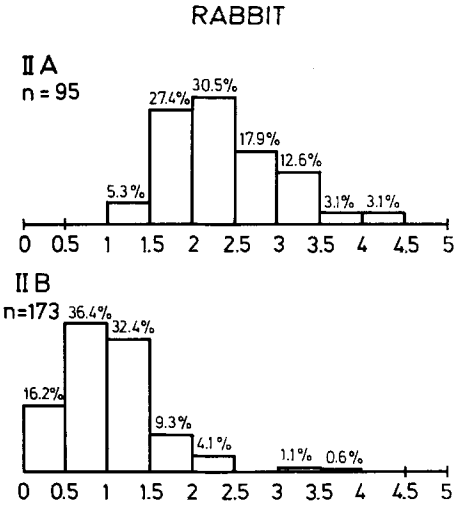

Fig. 7. Histograms of relative SDH activities in type IIA and IIB fibres of rabbit TA muscle

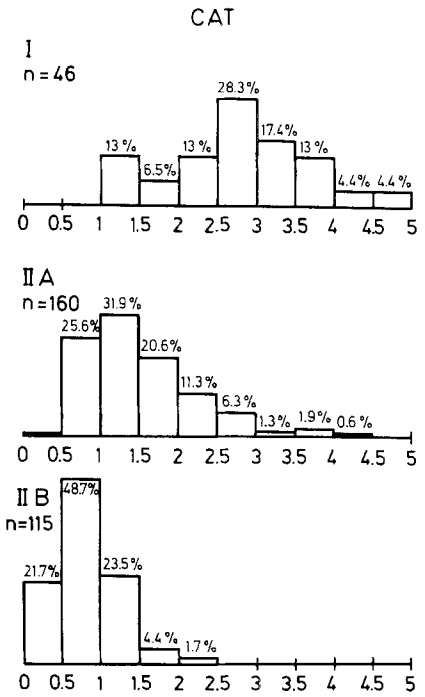

Fig. 8. Histograms of relative SDH activities in type I, IIA and IIB fibres of cat TA muscle

of guinea pig (Fig. 6), rabbit (Fig. 7) and cat (Fig. 8). The scattering of the IIA population is more pronounced than that of the IIB fibres, as is especially evident in the cat (Figs. 3 and 8). A large overlap of these two populations is seen in each of the three muscles, although the majority of the IIA fibres reveals higher activities than the IIB fibres. The pronounced overlap of these two populations seen in the cat (Figs. 3 and 8) appears to be due to the low activity range of a major fraction of the IIA fibres. The relatively high percentage of low activity IIA fibres in cat TA is emphasized by the activity profile of type I fibres in this muscle. Almost 80% of the IIA fibres in cat TA is in the range of 0.5–2 whereas 80% of the type I fibres reveal activities higher than 2. A similar activity pattern is seen in human TA (Fig. 9). The majority of human type I fibres (75%) has relative SDH activities higher than 2.5 whereas the majority of IIA fibres (85%) is in the activity range below 2.5. As judged from the scattering, the activity profiles of IIA and IIB fibre populations are almost the same in human TA (Fig. 9).

A bimodal distribution of SDH activity is suggested in Fig. 9 for the IIB fibres. In order to examine whether such a bimodal distribution is characteristic of human IIB fibres, additional analyses were performed on human vastus lateralis (VL) muscle. Results are presented in Fig. 10. The data resemble those shown for TA (Fig. 9), however, no bimodal distribution is seen for the IIB fibres in this muscle. On the other hand, the IIB population of VL displays a larger scattering than the IIA fibres. As in TA (Fig. 9), type I fibres reveal the highest activities and the major fraction of these fibres is clearly beyond the main fraction of the IIA population.

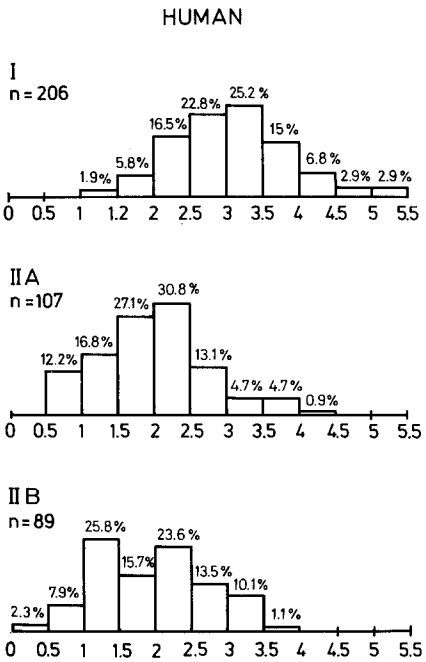

Fig. 9. Histograms of relative SDH activities in type I, IIA and IIB fibres of human TA muscle

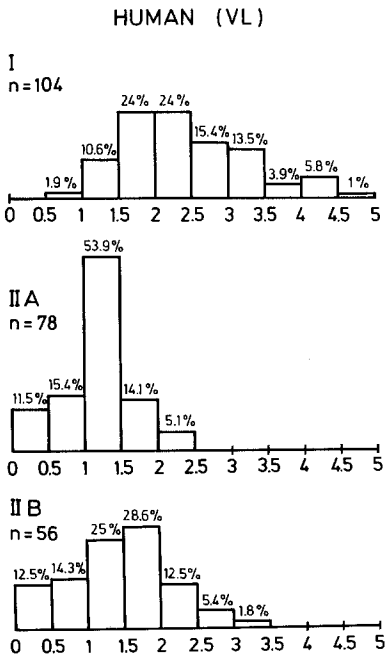

Fig. 10. Histograms of relative SDH activities in type I, IIA and IIB fibres of human vastus lateralis muscle

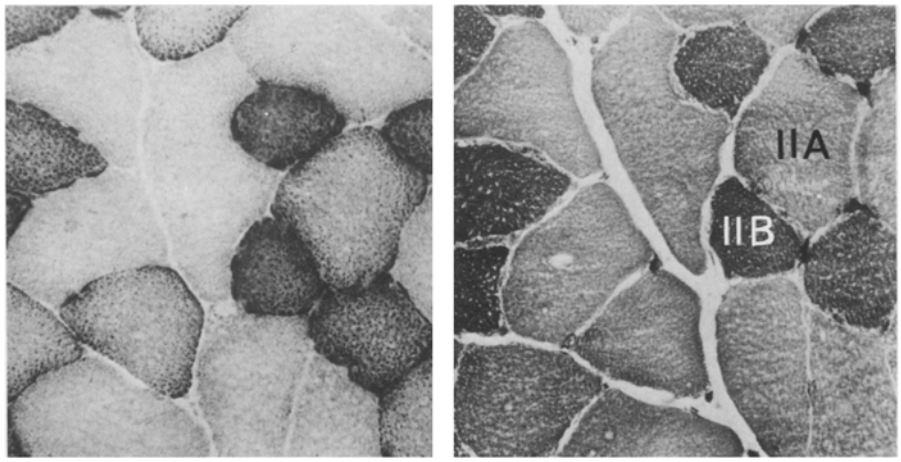

Fig. 11. Staining for SDH (left) and for myofibrillar actomyosin ATPase after preincubation at pH 4.6 in parallel sections of mouse TA muscle. × 240

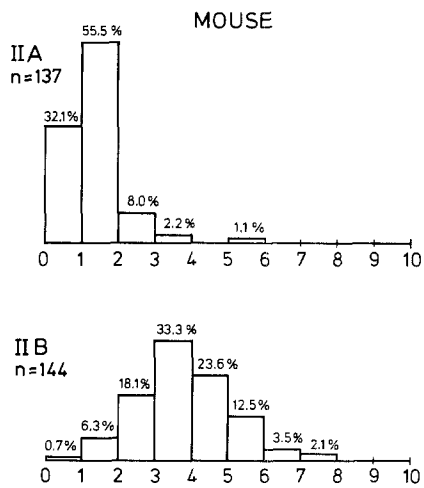

Fig. 12. Histograms of relative SDH activities in type IIA and IIB fibres of mouse TA muscle

The variability of SDH distribution in IIA and IIB fibres is emphasized by the results obtained in mouse muscle. As is evident from the staining intensities for SDH in Fig. 11, an inverse pattern of SDH distribution is characteristic of IIA and IIB fibres in the mouse. Type IIB fibres show a stronger reaction for SDH than the majority of the IIA fibres. Similar results as shown in Fig. 11 were obtained for TA muscles of various mouse strains (C 57 BL/6, C 3 H, and NMRI). According to qualitative enzyme histochemistry IIB fibres in mouse muscle could thus be classified as "oxidative", whereas IIA fibres with low staining for SDH as "glycolytic".

Microphotometric determinations of SDH activity confirmed these findings. The histograms of Fig. 12 illustrate that the main fraction of the IIB fibres is characterized by higher SDH activities than the IIA fibres. The main fraction

of IIA fibres reveals relative SDH activities below 2, whereas 90% of the IIB fibres has SDH activities above this value.

Discussion

In agreement with previous microphotometric determinations of SDH activity (Pette 1981; Pette and Tyler 1981; Nemeth and Pette 1981b) and microbiochemical activity determinations of other mitochondrial enzymes in microdissected single fibres (Spamer and Pette 1977, 1979, 1980; C. Lowry et al. 1978; Hintz et al. 1980; O. Lowry et al. 1980; Nemeth et al. 1981), large variations in SDH activity levels were observed in this study in type I, IIA and IIB fibre populations of TA muscles in various mammalians and the human. It can be ruled out that variations reaching the order of magnitude found in this study, might result from methodological errors. Measurements along fibres in serial sections indicated a limited variability and relatively high reproducibility (Table 2). As judged from the coefficient of variation, an average experimental error of less than 10% must be taken into account for comparative microphotometric determinations of SDH activity in serial muscle cross-sections. An experimental error of similar range applies to photometric measurements of SDH activity in tissue homogenates. Similarly, an experimental error in the range of 5%–10% has to be taken into account in microbiochemical enzyme activity measurements on microdissected tissue probes (e.g. Spamer and Pette 1977; Nemeth et al. 1981). An experimental error of less than 10% is remarkable in view of the specification of the applied microtechnique. It can be calculated that a microphotometric activity determination in a tissue cylinder of 16 µm diameter and 8 µm height corresponds to a sample of 0.5×10^{-9} g dry weight. This amount is about one tenth of the mean sample size used for microbiochemical enzyme measurements in pieces of microdissected single muscle fibres (Spamer and Pette 1977).

A prerequisite of comparative microphotometric activity determination in situ is that all measurements are taken within a measuring field of identical size. By using a round measuring field of 16 µm diameter, SDH activity was assessed only within interfibrillar mitochondria of the fibre core. Depending on the fibre type, a considerable fraction of the mitochondria is located within the subsarcolemmal zone. Due to the polygonal shape of muscle fibres, measurement of SDH activity in the subsarcolemmal region would create serious methodological problems which at the present level of instrumentation cannot be solved. Subsarcolemmal mitochondria which represent only a minor fraction of the total mitochondria in the muscle fibre, were therefore disregarded in the present study.

The results of this study support the view that it is impossible to classify muscle fibres by their metabolic properties in a system of a few fixed types. Using the myosin based classification of type I, IIA and IIB fibres, three populations may be distinguished. These populations, however, could not be discerned unambiguously according to their aerobic oxidative capacity as represented by the mitochondrial activity of SDH. Up to 10-fold variations in enzyme activity produce a large overlap in spectra between the various fibre types.

For all muscles studied, the scattering was found to be more pronounced in type II than in type I fibres. This confirms our previous results of microbiochemical enzyme measurements on single rabbit muscle fibres (Spamer and Pette 1977, 1979, 1980).

It thus appears evident that species differences exist for the aerobic oxidative capacity of the different fibre types. As originally stated by Peter et al. (1972), fast twitch (type II) fibres may vary markedly in their aerobic oxidative capacity, e.g. FG and FOG fibres. As is evident from the results of SDH activity measurements, no correlation exists, however, between this metabolic property and the myofibrillar actomyosin ATPase characteristics. Although it appears that there is a correspondence for the major fractions of IIA and IIB fibres with the FOG and FG types respectively in the rabbit, this is not valid in other species. The activity profiles of IIA and IIB fibres in rat and guinea pig TA muscles (Figs. 5 and 6) are characterized by large scattering and overlap. In the case of human muscle, there is an almost complete overlap of the two populations (Figs. 9 and 10). Finally in mouse TA muscle, the majority of the IIA fibres is in the range of low and the main fraction of the IIB fibres in the range of high SDH activity. It must be concluded therefore that no interchangeability exists for the two commonly used systems of fibre classification by Peter et al. (1972) and Brooke and Kaiser (1970).

It is obvious that the major fraction of type I fibres in cat TA (Fig. 8) and in the two human muscles (Figs. 9 and 10) reveal higher SDH activities than the majority of the IIA fibres. Higher average SDH activities in type I than in type IIA fibres have previously been reported on microdissected single fibres of human muscle by Essén et al. (1975). Conversely, lower average SDH activities in type I than in type IIA fibres were recently determined by kinetic microphotometry in rat muscle fibres (Nemeth and Pette 1981 b). It appears thus that species differences exist also for the aerobic oxidative capacity of type I fibres.

It cannot be assumed that the metabolic properties of a given fibre type are homogeneous within different muscles of the same animal. It was shown previously that pronounced differences may exist in activity levels of glycolytic and mitochondrial enzymes in fibres of the same type within different muscles of the rat and rabbit (Nolte and Pette 1972; Spamer and Pette 1977, 1978, 1980). Discrete differences seen in the distribution of SDH activity in IIB fibres of the two human muscles investigated in this study, may be explained that way.

Variations in the activity profiles of SDH may be explained to some extent by factors such as differences in overall motor activity or different patterns of motor unit recruitment. Among the different species studied, the caged laboratory rabbit evidently reveals the lowest spontaneous motor activity. It is not surprising that a major fraction of the IIB fibres in rabbit TA displays low SDH activity. It was shown in a recent study that these fibres respond to increased motor activity as induced by chronic indirect stimulation, with higher percentage increases in SDH than the IIA fibres (Pette and Tyler 1981). The observed scattering in SDH levels in all fibre populations may reflect differences in the neuronal activity of individual motor units. Neuronal input is an essential

factor in the regulation of metabolic properties. This has recently been substantiated by the finding that muscle fibres belonging to the same motor unit, display identical enzyme activities (Nemeth et al. 1980, 1981).

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References

Ariano MA, Armstrong RB, Edgerton VR (1973) Hindlimb muscle fibre populations of five mammals. J Histochem Cytochem 21:51–55

Brooke MH, Kaiser KK (1970) Three 'myosin ATPase' systems: The nature of their pH lability and sulfhydryl dependence. J Histochem Cytochem 18:670–672

Essén B, Jansson E, Henriksson J, Taylor AW, Saltin B (1975) Metabolic characteristics of fibre types in human skeletal muscle. Acta Physiol Scand 95:153–165

Hintz CS, Lowry CV, Kaiser KK, McKee D, Lowry OH (1980) Enzyme levels in individual rat muscle fibers. Am J Physiol 239:C58–65

Lowry CV, Kimmey JS, Felder S, Chi MM-Y, Kaiser KK, Passonneau PN, Kirk KA, Lowry OH (1978) Enzyme pattern in single human muscle fibres. J Biol Chem 253:8269–8277

Lowry OH, Lowry CV, Chi MM-Y, Hintz CS, Felder S (1980) Enzymological heterogeneity of human muscle fibres. In: Pette D (ed) Plasticity of muscle. W de Gruyter, Berlin New York, pp 3–18

Nemeth P, Pette D (1980) The interrelationship of two systems of fibre classification in rat EDL muscle. J Histochem Cytochem 28:193

Nemeth P, Pette D (1981a) The limited correlation of the myosin-based and the metabolic-based classifications of skeletal muscle fibres. J Histochem Cytochem 29:89–90

Nemeth P, Pette D (1981b) Succinate dehydrogenase activity in fibres classified by myosin ATPase in three hind limb muscles of rat. J Physiol (Lond) 320:73–81

Nemeth P, Hofer HW, Pette D (1979) Metabolic heterogeneity of muscle fibres classified by myosin ATPase. Histochemistry 63:191–201

Nemeth P, Pette D, Vrbová G (1980) Malate dehydrogenase homogeneity of single fibres of the motor unit. In: Pette D (ed) Plasticity of Muscle. W de Gruyter, Berlin New York, pp 45–54

Nemeth P, Pette D, Vrbová G (1981) Comparison of enzyme activities among single muscle fibres within defined motor units. J Physiol 311:489–495

Nolte J, Pette D (1972) Micorphotometric determination of enzyme activity in single cells in cryostat sections. II. Succinate dehydrogenase, lactate dehydrogenase and triosephosphate dehydrogenase activities in red, intermediate and white fibers of soleus and rectus femoris muscle of rat. J Histochem Cytochem 20:577–582

Ogawa K, Shinonaga V (1961) Role of intermediators in the trazolium histochemistry. Acta Anat Nippon 36:550–560

Peter JB, Barnard RJ, Edgerton VR, Gillespie CA, Stempel KE (1972) Metabolic profiles of three fibre types of skeletal muscle in guinea pigs and rabbits. Biochemistry 11:2627–2633

Pette D (1981) Microphotometric measurement of initial maximum reaction rates in quantitative enzyme histochemistry in situ. Histochem J 13:319–327

Pette D, Tyler KR (1981) Quantitative changes in oxidative enzyme activity during chronic stimulation of rabbit fast muscle. J Physiol (Lond) 317:22P

Pette D, Wimmer M (1980) Microphotometric determination of enzyme activities in cryostat sections by the gel film technique. Trends in Enzyme Histochemistry and Cytochemistry. Ciba Foundation Symposium 73 (new series). Excerpta Medica, Amsterdam Oxford New York, pp 121–134

Pette D, Wasmund H, Wimmer M (1979) Principle and method of kinetic microphotometric enzyme activity determination in situ. Histochemistry 64:1–10

Pette D, Wimmer M, Nemeth P (1980) Do enzyme activities vary along muscle fibres? Histochemistry 67:225–231

Spamer C, Pette D (1977) Activity patterns of phosphofructokinase, glyceraldehydephosphate dehydrogenase, lactate dehydrogenase and malate dehydrogenase in microdissected fast and slow fibres from rabbit psoas and soleus muscle. Histochemistry 52:201–216

Spamer C, Pette D (1979) Activities of malate dehydrogenase, 3-hydroxyacyl-CoA dehydrogenase and fructose-1,6-diphosphatase with regard to metabolic subpopulations of fast- and slow-twitch fibres in rabbit muscles. Histochemistry 60:9–19

Spamer C, Pette D (1980) Metabolic subpopulations of rabbit skeletal muscle fibres. In: Pette D (ed) Plasticity of muscle. W de Gruyter, Berlin New York, pp 45–54

Spurway NC (1980) Histochemical typing of muscle fibres by microphotometry. In: Pette D (ed) Plasticity of muscle. W de Gruyter, Berlin New York, pp 31–44

Spurway NC (1981) Interrelationship between myosin-based and metabolism-based classifications of skeletal muscle fibre. J Histochem Cytochem 29:87–88

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