The ratio between intrinsic 115 kDa and 30 kDa peptides as a marker of fibre type-specific sarcoplasmic reticulum in mammalian muscles

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Sarcoplasmic reticulum displays characteristic differences in Ca^{2+}-uptake, Ca^{2+}-ATPase and the pattern of membrane proteins in type I, IIA and IIB fibres. The ratio between the 115 kDa Ca^{2+}-ATPase and a 30 kDa protein is of characteristic magnitude in the sarcoplasmic reticulum of the three fibre types in rat muscles. The slow-to-fast fibre type transformation observed in rabbits during chronic nerve stimulation is accompanied by predictable changes of this ratio.

Sarcoplasmic reticulum *Muscle fibre type I, IIA, IIB* *Intrinsic membrane protein* *Chronic stimulation*

1. INTRODUCTION

It is now generally accepted that slow- and fast-twitch mammalian muscles, in addition to their different isotypes of contractile and regulatory proteins, differ in the content [1] and properties [2–8] of their sarcoplasmic reticulum (SR). These differences refer mainly to muscles that are composed of either type I (slow) or type II (fast) fibres. However, IIA and IIB subtypes of the fast fibre population differing in metabolic properties [9], coexist in most fast-twitch muscles. We were interested in studying the properties of the SR isolated from muscles that are composed mainly of one of these 3 fibre types. Characteristic SR species were found to exist in the 3 fibre types according to Ca^{2+}-uptake, Ca^{2+}-dependent ATPase activities, peptide patterns, and the ratios of defined peptides, especially that between the 115-kDa Ca^{2+}-ATPase and a 30-kDa peptide. Fibre transformation as induced by chronic nerve stimulation led to changes in each of these parameters.

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2. METHODS

SR was isolated from superficial (VLS, predominantly type IIB) and deep (VLD, predominantly type IIA) vastus lateralis and soleus (SOL, predominantly type I) muscles of adult male Wistar rats as in [10]. Methods for measuring Ca^{2+}-uptake and Ca^{2+}-dependent ATPase at 37°C were as in [10]. Electrophoresis was performed according to [11] as described [10] and the peaks were evaluated densitometrically (Ultroscan Laser Densitometer, LKB) after staining with Coomassie blue. Chronic (24 h/day) nerve stimulation (10 Hz) of fast-twitch tibialis anterior (TA) and extensor digitorum longus (EDL) muscles of the rabbit (white New Zealand strain) was performed as in [10].

3. RESULTS AND DISCUSSION

There are pronounced differences in the electrophoretically separated peptide components of the SR preparations from the 3 fibre types (fig.1). Type IIB fibres contain the highest amounts of 115-kDa Ca^{2+}-ATPase and calsequestrin when

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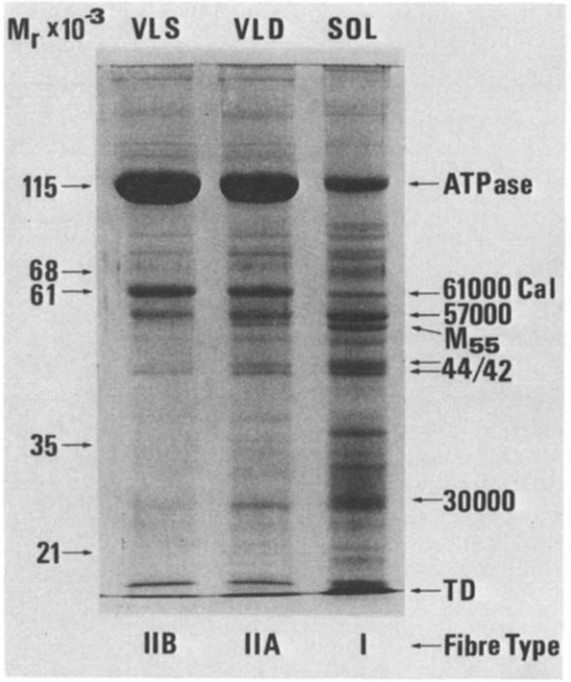

Fig.1. Electrophoresis according to [11] of sarcoplasmic reticulum from 3 rat muscles differing in fibre composition. Superficial (VLS) and deep (VLD) vastus lateralis muscle are rich in fibre types IIB and IIA, respectively. Soleus muscle (SOL) contains predominantly type I fibres.

compared to types IIA and I. Conversely, a 30-kDa peptide which is most prominent in type I, is found at lower and lowest concentrations in types IIA and IIB, respectively. Thus, the ratio 115-kDa/30-kDa peptides varies greatly between the 3 fibre types. Its value is 14.1 ± 3.5, 3.8 ± 0.8 and 1.2 ± 0.4 for types IIB, IIA and I, respectively. The 30-kDa peptide has been described as an integral membrane protein characteristic of SR in slow-twitch muscle and has been tentatively identified as NADH-cytochrome b_5 reductase [12]. In [13] an increase of a 30-kDa peptide was observed in SR of denervated fast-twitch muscle of the chicken.

Further differences between the SR of the 3 fibre types are related to the content of a 55-kDa peptide (fig.1). This peptide was tentatively identified as M_{55}-high affinity Ca^{2+} binding protein. Its colour reaction with the stains-all dye after one- and two-dimensional electrophoresis (not shown) excluded the possibility that it was either calsequestrin or the 53-kDa glycoprotein described in [14]. Increased amounts of this compound were also observed in denervated muscle [13]. Significant differences exist between SR of the 3 fibre types in both the initial and maximum Ca^{2+}-uptake (fig.2).

The fibre-type specificity of the SR could be further confirmed by experimentally induced fibre type transformations. Chronic nerve stimulation of fast-twitch muscles with a low frequency (10 Hz) leads to a virtually complete fast to slow transformation of the SR with regard to Ca^{2+}-uptake, Ca^{2+}-ATPase, phosphoprotein formation, peptide pattern and ultrastructure [10,15,16]. The time course of the changes in the peptide pattern of the SR is plotted in fig.3. The two muscles under study (TA and EDL) contain <5% type I and ~60% IIB fibres. Chronic stimulation induced pronounced decreases both in

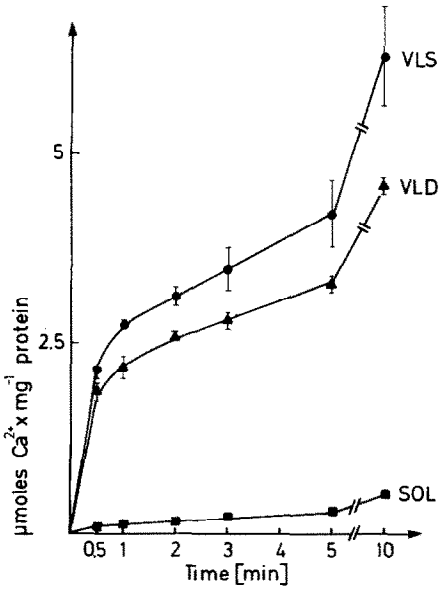

Fig.2. Time course of Ca^{2+}-uptake in the presence of 10 mM oxalate and 0.2 mM $^{45}CaCl_2$ by sarcoplasmic reticulum protein (100 μg) isolated from muscles which are predominant in fibre types IIB (VLS), IIA (VLD) or I (SOL). Initial uptake is 4.3, 3.6 and 0.23 μmol $Ca^{2+}.min^{-1}.mg\ protein^{-1}$ and maximum uptake after 10 min is 6.2 ± 0.6, 4.5 ± 0.1 and 0.53 μmol Ca^{2+}/mg protein for SR of VLS, VLD and SOL, respectively. Ca^{2+}-dependent ATPase activities of the same preparations are 2.31 ± 0.13, 1.87 ± 0.04 and 0.18 U/mg protein.

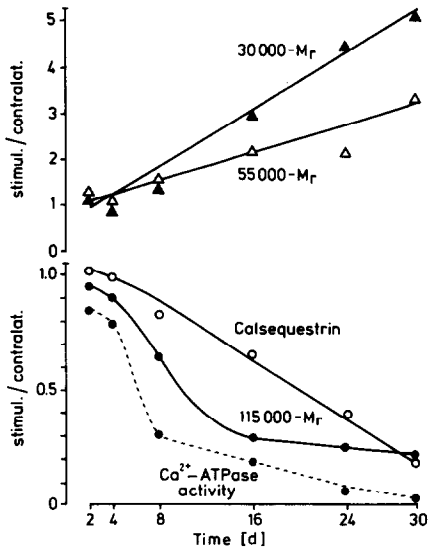

Fig.3. Time course of electrophoretically determined specific peptides of SR from fast-twitch rabbit muscle during chronic nerve stimulation. Values are expressed as ratios between stimulated and contralateral muscles.

115-kDa Ca^{2+}-ATPase and 61-kDa calsequestrin and also marked increases both in 55-kDa and 30-kDa peptides. As judged from the peptide patterns and the 115-kDa/30-kDa ratios given in table 1, the transformation of the SR is completed after a 3 week stimulation period. This ratio has changed already after 16–20 days to that determined for slow-twitch soleus muscle (100% type I) of the rabbit. The transformation of the SR, therefore, occurs well in advance of the exchange of fast-to-slow type myosin [17,18].

Table 1

Changes in the ratio of intrinsic membrane proteins of sarcoplasmic reticulum during chronic stimulation of fast-twitch rabbit muscles (the value of slow-twitch soleus muscle is given for comparison)

TA and EDL	115-kDa Ca^{2+}-ATPase/ 30-kDa peptide
Unstimulated	12.5 ± 4.3 ($n = 6$)
Stimulated	
2 days	16.5
4 days	10.3
8 days	5.4
16 days	0.9
24 days	0.5
30 days	0.4
Soleus	0.7

The 115-kDa/30-kDa peptide ratio appears to be a highly sensitive marker of fibre-type specific differences in the SR. Taking into account the values of this ratio determined in muscles that contain mainly IIB and IIA fibres in the rat (see above), the decrease of the ratio during stimulation of rabbit fast-twitch muscles (mainly IIB) would suggest a sequence of fibre transformation from IIB to IIA to I. Such a sequence has recently been suggested from myosin changes occurring during long-term intermittent (8 h/day) stimulation [19]. In [13] a decrease was demonstrated in the ratio between 100-kDa and 30-kDa peptides from 16, the value normally observed, to 3 in the denervated fast-twitch muscle of the chicken. This change may be interpreted as a fast to slow transformation. As a matter of fact, such a transformation has previously been shown to occur in the denervated fast-twitch rat muscle as judged by changes in Ca^{2+}-uptake and Ca^{2+}-dependent ATPase [5].

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