The effect of cross-innervation on the tropomyosin composition of rabbit skeletal muscle

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Soleus, semitendinosus and crureus muscles of the rabbit were found to contain α- and β-tropomyosin subunits and additional forms that have been provisionally designated γ and δ. Extensor digitorum longus and psoas muscles contained only α and β subunits, the relative proportions of which varied between single fibres of psoas muscle. On cross-innervation of rabbit soleus and extensor digitorum longus muscles, the fraction of the total tropomyosin present as the β subunit remained constant. The relative proportions of α, γ and δ subunits changed as would be expected from the change in speed that occurred.

Rabbit skeletal muscle *Tropomyosin isotype* *Tropomyosin phosphorylation* *Cross-innervation* *Single muscle fibre*

1. INTRODUCTION

It is now well documented that the expression of the genes controlling the synthesis of the various isotypes of most of the myofibrillar proteins in skeletal muscle is affected by the frequency of stimulation of the muscle. These effects have been demonstrated both after cross-innervation and direct electrical stimulation for myosin [1–6], and after cross-innervation for the components of the troponin complex [7,8]. The position regarding tropomyosin is not so clear cut. In the original studies it was concluded from the appearance of 1-dimensional electrophoretic gels of tropomyosin isolated from rabbit muscles that the relative amounts of the α and β subunits had not significantly altered after cross-innervation of the soleus muscle with the lateral popliteal nerve [7]. In the same muscles troponin I had changed from the mainly slow to the mainly fast isotype. Later studies, in which fibre type changes in soleus and extensor digitorum longus muscles after cross-innervation were investigated using peroxidase-labelled antibodies, indicated that tropomyosin had undergone isotypic changes similar to those observed in the troponin complex [8]. Also in experiments with chronically-stimulated rabbit muscles, changes in the electrophoretic pattern of the tropomyosin compatible with expected fibre type change have been reported [9].

As there is evidence that during development the factors affecting the expression of the tropomyosin genes may be different from those associated with the isotypes of the other myofibrillar proteins [10], it is conceivable that a similar situation applies during cross-innervation. In view of this possibility, the changes occurring in tropomyosin during cross-innervation of skeletal muscle have been re-investigated using the resolving power of 2-dimensional electrophoresis.

2. MATERIALS AND METHODS

2.1. *Physiological procedures*

The muscles analysed were those obtained in earlier studies on the effects of cross-innervation of leg muscles of New Zealand red rabbits [8] and which had been stored frozen at −70°C. They

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consisted of samples of soleus and extensor digitorum longus muscles that had been cross-innervated with the lateral popliteal and soleus nerves, respectively, for periods up to 28 weeks and their contralateral controls. A thin transverse slice had previously been removed from the centre of each muscle for cell typing.

2.2. *Electrophoresis of muscle samples*

Control and cross-innervated whole muscle samples were homogenized in 10 vol. (w/v) of 9 M urea and the solution then made 15 mM with respect to β-mercaptoethanol and 2% (v/v) with respect to the detergent, NP40. The homogenate was immediately subjected to isoelectric focusing and 2-dimensional electrophoresis as in [11] using the procedures in [12,13]. The pH range for the isoelectric focusing dimension was from 4–6, using 2% ampholine (LKB Instruments, Croydon). The relative amounts of the tropomyosin subunits were determined by densitometric scanning of the gels as described earlier [14].

2.3. *Single fibres*

The crureus, soleus, semitendinosus and psoas muscles were excised from rabbits and immersed in 50% glycerol at −25°C for 3–4 weeks. Single fibres were dissected from bundles of fibres under a dissection microscope and homogenized in 10 μl of 9 M urea using a microhomogenizer of the pestle type. The sample was subjected to 2-dimensional electrophoresis as described, using a second dimension gel of 0.5 mm thickness which was stained using the silver method in [15].

3. RESULTS

Electrophoresis of extracts of whole extensor digitorum longus (EDL) muscle indicated that normal adult muscle contained only α and β subunits in roughly equal amounts with 10–20% of the α subunit as the phosphorylated form (fig.1A, table 1). This is in agreement with the results in [16], whereas authors in [9] reported much higher α/β ratios in control EDL muscles of the rabbit in their studies on direct stimulation [9]. The subunit composition of the tropomyosin from soleus, semitendinosus and crureus muscles was more complex, consisting of α and β subunits with additional components which we have preliminary designated as γ and δ subunits (fig.1a). The γ and δ subunits may represent further resolution of the additional spot recently reported to be associated with α and β tropomyosin in the human [17], rabbit [18] and the cat [19]. These latter components are considered to represent tropomyosin subunits on the basis of the following evidence:

(1) They co-purified with α- and β-tropomyosin;
(2) Their electrophoretic mobilities changed in the anomalous manner unique to tropomyosins, when urea is present in addition to sodium dodecyl sulphate [20,21];
(3) Radioactive forms of α-, β- and γ- or δ-tropomyosin were detected when slices of crureus muscle were incubated with Eagles Medium containing ^{32}P-labelled inorganic phosphate. The extent of phosphorylation of the γ or δ subunits was found to change during development of crureus muscle, a feature that is characteristic of tropomyosin [14,22].

Single type I fibres identified by the electrophoretic pattern of the myosin light chains were found to contain the α, β, γ and δ subunits in relative proportions similar to those observed in extracts of whole muscle.

It is possible that some variation in relative amounts of the subunits occurred between different type I fibres in slow muscle, but not enough individual fibres were examined to come to a conclusion on this point. Single fibres from rabbit psoas muscle which consists mainly of type II cells, however, did vary in the relative proportions of β-tropomyosin present. In some fibres β-tropomyosin could not be detected on silver-stained electropherograms, whereas in others it represented about 30–40% of the total (fig.1B,vi,vii). Neither γ or δ subunits could be detected in fibres from psoas muscle.

After 8 weeks cross-innervation of the soleus muscle, the relative proportions of the tropomyosin subunits were found to change. The γ and δ subunits were much reduced in amount and an elevated level of the α subunit was observed (fig.1B,ii). After 28 weeks, the transformation of the soleus muscle to a fast muscle, as assessed by measurement of time to peak tension, was complete [8]. At this stage, only traces of γ and δ subunits were present and the α and β subunits were present in a ratio very similar to that found in EDL muscle (table 1, fig.1B,iii). On cross-

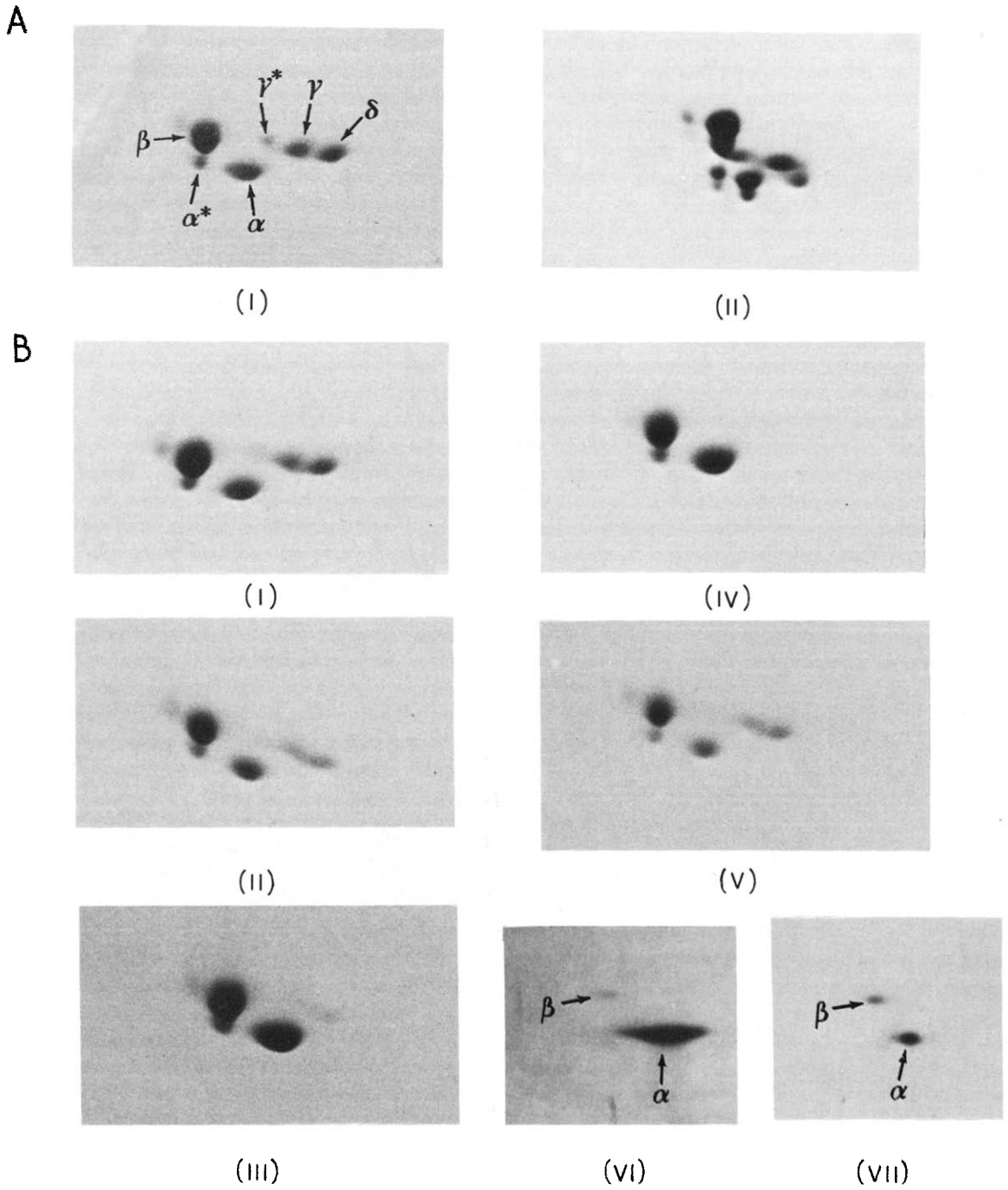

Fig.1. Tropomyosin subunits of control and cross-innervated rabbit skeletal muscles. Only regions of 2-dimensional electropherograms occupied by tropomyosin subunits shown: (A) Normal muscles: (i) Soleus, spots labelled to illustrate different forms. An asterisk indicates the phosphorylated form of the subunit. In the case of the phosphorylated γ subunit the identification is provisional; (ii) Crureus, electropherogram with improved M_r-resolution showing two forms of the α and β subunits. (B) Cross-innervated muscles and single fibres: (i) Soleus, control; (ii) Soleus, 8 weeks cross-innervated; (iii) Soleus, 28 weeks cross-innervated; (iv) EDL control; (v) EDL, 22 weeks cross-innervated; (vi) and (vii) Single fibres of psoas muscle at different loadings to illustrate the different relative amounts of α and β subunits.

Table 1

Changes in cell type and tropomyosin subunits during cross-innervation of rabbit soleus and extensor digitorum longus muscles

Muscle	Time cross-innervated (weeks)	Time to peak (ms)	Total cells (%)[a] Type II (%)	Type I (%)	Inter-mediate (%)	Tropomyosin subunits (% of total) α	β	$\gamma + \delta$
Soleus	0	80	0.0	76.0	24.0	31	46	23
	8	40	15.7	20.7	63.6	36	51	13
	28	27	92.2	7.1	0.6	45	49	6
Extensor digitorum longus	0	25	92.0	8.0	0.0	46	54	0
	22	50	14.0	85.0	1.0	31	52	17

[a] Data from [8]

Type I and type II cells are those cells that stained for slow and fast troponin I, respectively. Intermediate cells stained for both the fast and slow forms of troponin I. The amounts of the tropomyosin subunits are expressed as percentages of the total tropomyosin as estimated from densitometric tracings of isoelectric focusing gels. These results were found to be comparable with similar studies in which separation was carried out on 2-dimensional gels

innervation of EDL muscle, changes in tropomyosin composition complementary to those occurring in soleus muscle were observed (fig.1B,iv,v). It was noted, however, that although the relative amounts of α, γ and δ subunits changed as a consequence of cross-innervation, the amount of β subunit as a percentage of the total tropomyosin did not. The average value for the percentage of β subunit from all the control and cross-innervated soleus and EDL muscles was 48.8 ± 0.56 ($n = 17$). These results suggest that the differences in the tropomyosin composition between normal soleus and EDL muscles was in the relative amounts of α, γ and δ subunits. The total fraction of the tropomyosin represented by these 3 subunits was very similar in all muscles examined. It follows, therefore, that the transformation of fibre types associated with cross-innervation involves marked changes in the expression of the genes controlling the α, γ and δ subunits, but not those controlling the β subunits. Some caution should be expressed in coming to this conclusion as the tropomyosin subunit composition may be more complex than is apparent. For example, with improved electrophoretic separation in the M_r dimension [23], α and β subunits appeared to migrate as a poorly resolved doublet (fig.1A,ii).

In the light of the results presented here it is now possible to explain why in an earlier study [7], the tropomyosin composition of soleus muscle appeared unchanged after cross-innervation. On the 1-dimensional electrophoretic system used in the original study, changes in the proportions of the α, γ and δ subunits were not observed as these subunits all migrated in the unresolved α band. As the relative amount of β subunit is similar in fast and slow muscles, no significant change in the α/β ratio obtained from 1-dimensional electrophoretic analysis could be observed after cross-innervation.

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