Characterization of a Ventricular V1 Myosin Isoform in Rabbit Masticatory Muscles. Developmental and Neural Regulation

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Abstract

The masseter and retractor mandibulae of adult rabbits were shown by electrophoresis of native myosin to contain a trace of the slow-type I isoform, about 50% of the fast-type II isoforms, and about 50% of an isoform having an electrophoretic mobility intermediate between those of the slow and fast isoforms. This atypical myosin isoform migrated just ahead of the rabbit cardiac auricle A1 isoform. Its ATPase activity measured on gel or on muscle cross-section was high and both acid- and alkali-stable. It contained α-type cardiac heavy chains and ventricular-type light chains. It was characterized as a ventricular V1 myosin isoform.

The V1 isoform appeared in the masticatory muscles during the third week after birth, later than the type II isoforms. The proportion of V1 increased progressively, reaching a maximum of 80% at around three months, and then decreased to a stable value of 50%. This variation with age of the proportion of V1 is reminiscent of its variation in the cardiac ventricles [Lompré AM et al.: *Dev. Biol.* 1981; 84: 286-290]. Denervation of the retractor mandibulae induced the inhibition of the synthesis of V1.

Key words: Cardiac myosin, myosin isoforms, masticatory muscles, development, innervation.

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Masticatory muscles appear to be highly specialized in mammals, exhibiting different contractile properties in small rodents, ruminants, and carnivorous predators. These physiological differences probably result from differences in the fibre composition of the muscles, and in particular from differences in their myosin isoform content.

The masseter muscle has been the object of most of the studies concerning masticatory muscles. The masseter of small rodents (mouse, rat, guinea-pig) is uniformly composed of fast-twitch type II fibres [29, 33], containing fast-type myosin isoforms [11], well adapted to a powerful or sudden activity for biting or gnawing. The masseter of ruminants (cattle, sheep) consits, on the other hand, of only slow-twitch oxidative type I fibres [33], containing the slow-type myosin isoform [37]. This is in good correlation with the functional demand in ruminants for slow, steady cud chewing. The masseter and the temporalis of predators (cat, dog), which contract more rapidly than the limb and body fast muscles, have been shown to consist of a new type of fibre, the superfast type IIM fibre, containing a superfast myosin isoform, which is immunologically and biochemically different from limb muscle myosin [26, 30, 16].

The masseter muscle of the rabbit has also been the object of a number of investigations, but the results concerning this mammal are less clear-cut. The muscle is heterogeneous [29, 20, 24], and histochemical typing has revealed a regional distribution of six fibre types, mainly fast- and slow-twitch oxidative types [5]. Fast-type myosin isoforms are present, as well as an unusual slow-type myosin isoform [28, 3, 20, 14]. On the basis of immunocytochemical results, Bredman *et al.* [4] have proposed recently that the rabbit masseter also contains a "cardiac specific" α myosin heavy chain.

In the present study, we report the presence of a ventricular V1 cardiac-type myosin isoform in three masticatory muscles of the rabbit, the masseter, the retractor mandibulae, and the temporalis. In addition, we describe the developmental and neural regulation of the V1 isoform.

Materials and Methods

Muscles

New-Zealand rabbits were provided by Lessieux and CEGAV. The retractor mandibulae and masseter muscles of newborn, young, adult, and old animals were carefully dissected and immediately frozen in liquid nitrogen for biochemical studies and in isopentane cooled in liquid nitrogen for histochemical studies. For adult animals, the masseter was sometimes separated into three pieces, corresponding to the posterior deep masseter, to the anterior deep and middle masseter, and to the superficial masseter [36]. In addition to the retractor mandibulae and masseter, the temporalis, gastrocnemius medialis, soleus, and diaphragm, as well as the heart auricles and ventricles, were also dissected for compari-

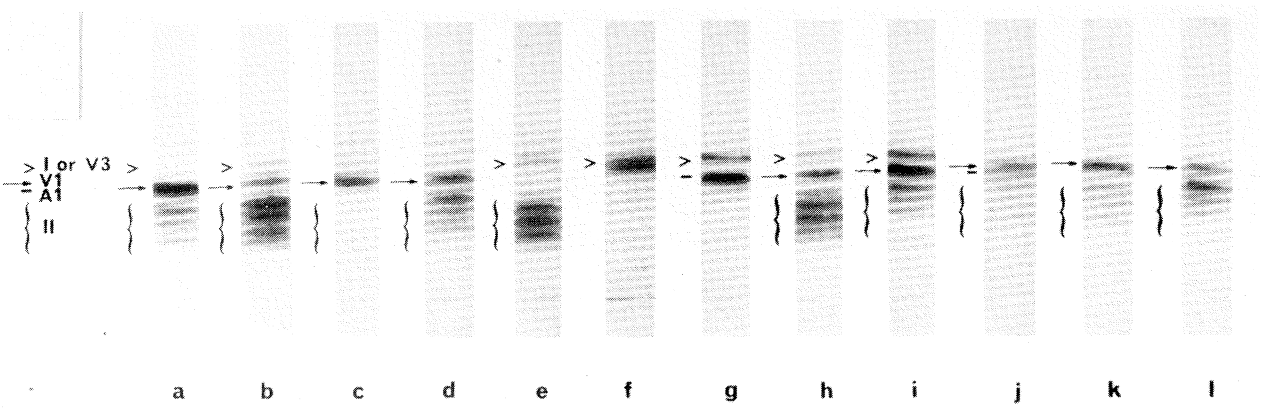


Figure 1 Electrophoresis under non-dissociating conditions of the myosin isoforms present in various adult rabbit muscles. a: masseter (whole muscle); b: posterior deep masseter; c: anterior deep and middle masseter; d: superficial masseter; e: gastrocnemius medialis; f: soleus (this muscle displays a doublet corresponding to two type I myosins, SM, which is here designated by >, and SM', which runs just behind SM and which is not present in the other muscles examined in this study); g: cardiac ventricles and auricles; h: gastrocnemius medialis + masseter; i: ventricles + masseter; j: auricles + masseter; k: retractor mandibulae; l: temporalis. The symbols on the left designate the different isoforms. > slow type I isoform SM or ventricular isoform V3; ---> ventricular isoform V1; -auricular isoform A1; { type II isoforms.

son.

The right retractor mandibulae was denervated by section of the bundle of nerves innervating the muscle, on 8-day- and 1-month-old (just after weaning) rabbits. The effect of denervation was examined at different times. The sural triceps of the right leg of the same animals was also denervated by sectioning the nerve innervating the gastrocnemius and soleus muscles.

Antibodies

A monoclonal antibody (249-5A4) specific to the α-type cardiac myosin heavy chain was a generous gift of Dr Moorman and Mr Wessels. Two monoclonal antibodies specific to the fast-type myosin were obtained from Sigma and Biomakor.

Myosin preparation

Muscles were thawed on ice, cut into small pieces, and homogenized with 5 vol. 20 mM NaCl, 5 mM sodium phosphate, and 1 mM EGTA (pH 6.5). Myosin was then extracted with 3 vol. 100 mM sodium pyrophosphate, 5 mM EGTA, and 1mM dithiothreitol (pH 8.5); after 30 min of gentle shaking, the mixture was centrifuged at 10,000 x g. The supernatant containing myosin was diluted twice with glycerol and stored at -20°C [12, 11].

Electrophoresis of native myosin isoforms. ATPase activity. Immunological blotting.

Gel running buffer consisted of 20 mM sodium pyrophosphate (pH 8.5), 10% glycerol, 0.01% 2-mercaptoethanol and 2 mM $MgCl_2$. Cylindrical (6 x 0.5 cm) and plate (6 x 6 cm) gels contained 4% polyacrylamide (3.88% acrylamide and 0.12% BIS). Between 1 and 5 μg of myosin was loaded on each gel. Electrophoresis was carried out at a constant voltage of 90 V, for 22 to 24 h, between 0 and 2°C [12, 11]. Gels were stained with Coomassie blue R-250, and the relative amounts of the different myosin isoforms were measured with a densitometer equipped with an integrator.

To determine the myosin Ca^{2+} ATPase activity of the separated isoforms, the pyrophosphate running buffer was replaced by ATP (5 mM) during the 2 last hours of the electrophoresis. The cylindrical gels were then loaded into tubes containing the ATPase assay buffer ($CaCl_2$ 15 mM, ATP 5 mM, pH 8.8). The appearance of white precipitates of calcium phosphate, reflecting the hydrolysis of ATP, was measured over time [17]. The effect of acidic preincubation was examined by incubating the gels for 30 min in a sodium acetate buffer (pH 4.3), rinsing them for 30 min at pH 8.8 in the ATPase assay buffer devoid of ATP and calcium, then incubating the gels in the ATPase buffer at pH 8.8 and proceeding as above.

The immunologic reaction of native myosin isoforms with specific anti-α and anti-fast myosin antibodies was determined by separating the isoforms on plate gels (60 V, for 20 h) and transferring them from the gels onto nitrocellulose sheets [35]. The primary antibodies were visualized with a peroxidase - labelled second antibody (goat anti-mouse IgM from Pasteur Production), followed by development with diaminobenzidine or chloronaphtol.

Two-dimensional gel electrophoresis of myosin light chains

Isoelectric focusing was performed by the technique described by O'Farrell [21], using a narrow pH range (4.5 - 5.5) for higher sensitivity. Myosin samples were diluted with 9.5 M urea, 1% Servalytes pH 4.5 - 5.0, 1% Servalytes pH 5.0 - 5.5, 2% Nonidet P-40 and 5% 2-mercaptoethanol and loaded on cylindrical gels (10 x 0.27 cm) containing the same servalytes. Focusing was carried out for 16 h at 400 V and 1 h at 800 V. The gels were then equilibrated in 10% glycerol, 5% 2-mercaptoethanol, 2.3% SDS, and 62.5 mM Tris-HCl (pH 6.8) and applied on polyacrylamide slab gels containing 15% acrylamide and 0.087% bisacrylamide. Electrophoresis was performed in the presence of SDS according to Laemmli [18] and silver nitrate staining according to Ansorge [1]. The light chains from individual myosin isoforms were analyzed by first separating the isoforms in their native state and by loading the gel bands containing each isoform on isoelectric focusing gels.

SDS-glycerol gel electrophoresis of myosin heavy chains. Immunological blotting.

Myosin heavy chains were separated in 5% polyacrylamide slab gels, in the presence of 0.1% SDS and 37.5% glycerol [7]. Electrophoresis was carried out at 120 V for 20 h. Reaction with specific antibodies was performed as above.

Histochemistry

Serial muscle cross-sections (10 μm) were incubated at pH 9.4 to measure muscle fibre Ca^{2+}-activated adenosine triphosphatase, either directly or after preincubation at pH 4.3 [15].

Results

CHARACTERIZATION OF A CARDIAC MYOSIN ISOFORM IN THREE MASTICATORY MUSCLES OF THE ADULT RABBIT

Analysis of the cardiac myosin in its native form. Electrophoretic mobility, immunologic reactivity, and ATPase activity.

The adult rabbit masseter was shown to contain 6 myosin isoforms, which separated by electrophoresis under non-dissociating conditions (Fig. 1a). By order of increasing electrophoretic mobilities, these were the type I SM slow isoform, a yet unidentified isoform which we will call cardiac, and four type II isoforms. The SM and the fourth type II isoforms were present in very small proportions (< 1%). Their presence was more clearly revealed by the analysis of the posterior deep portion of the muscle, which displayed the quasi-totality of the muscle content in these isoforms (Fig. 1b). The anterior deep and middle portions of the muscle, on the other hand, contained the cardiac isoform almost exclusively (Fig. 1c). The superficial masseter contained mixtures of the cardiac and type II isoforms (Fig. 1d).

To identify the unknown myosin isoform present in the masseter, its migration was compared with that of the isoforms present in various muscles of the adult rabbit: the gastrocnemius medialis, the soleus, and the heart ventricles and auricles (Fig. 1e - j). The gastrocnemius medialis contained SM and the four type II isoforms; the soleus contained a doublet corresponding to SM and SM'; the ventricles and the auricles contained the isoforms V3 and A1, respectively. The results of various comigration experiments indicated that the masseter cardiac isoform migrated at a position close to the position of A1, though somewhat ahead of it.

Several rabbit skeletal muscles were examined for the presence of a cardiac isoform; such an isoform was found in two other muscles involved in mastication, the retractor mandibulae and the temporalis (Fig. 1k and l). It was also found as a minor component in the tongue of the adult rabbit and transiently in the diaphragm of 2 to 3 week-old rabbits (not shown).

In its native state, the cardiac isoform did not cross-react with an anti-fast myosin antibody; it was shown, on the other hand, to react with an anti-α myosin antibody, specific to the auricle A1 myosin. (Fig. 2)

The Ca^{2+}-ATPase activity and pH lability of the cardiac myosin were compared to those of the type I and II myosins, respectively (Fig. 3). The type I isoform was completely inactive at pH 8.8, whereas the type II isoforms displayed ATPase activity, which was revealed on the gel by the progressive appearance of a calcium phosphate band. The cardiac isoform present in the rabbit masticatory muscles was shown in this study to display a specific ATPase activity, which was even higher than that of the type II isoforms. After preincubation at pH 4.3, the type II isoforms were almost inactivated, while the cardiac isoform remained active.

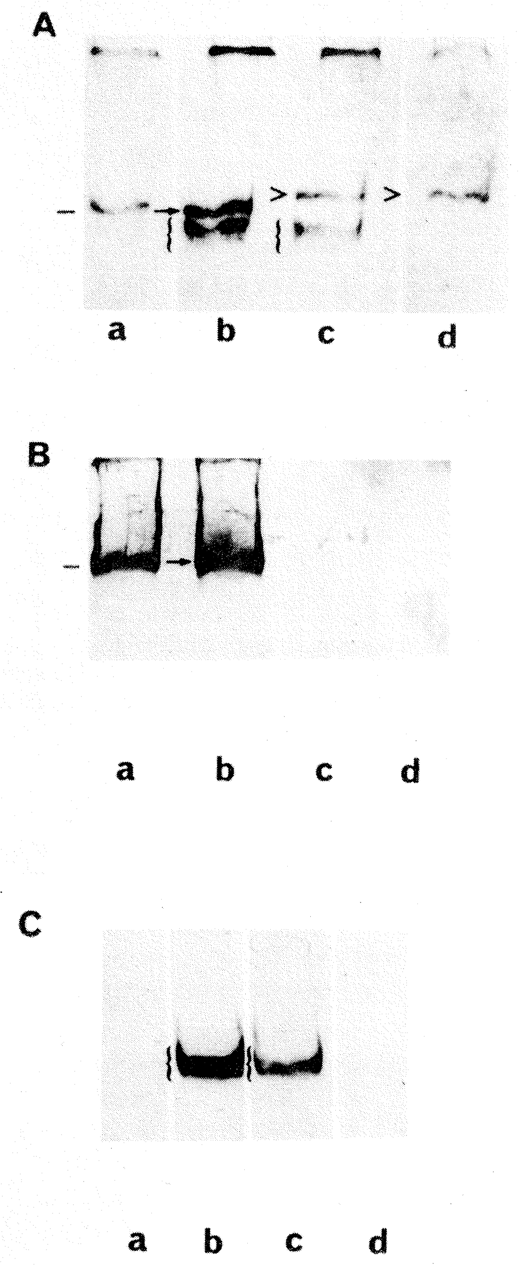


Figure 2 Electrophoresis under non-dissociating conditions and immunoblotting of the myosin isoforms present in various adult rabbit muscles. a: cardiac auricles; b: retractor mandibulae; c: diaphragm; d: cardiac ventricles; A: protein staining; B: cross-reaction with anti-α cardiac myosin heavy chain antibody (249-5A4); C: cross reaction with an anti-fast myosin antibody (Biomakor). The symbols designate the different isoforms. > ventricular isoform V3 or slow type I isoform SM; ---> ventricular isoform V1; - auricular isoform A1; { type II isoforms

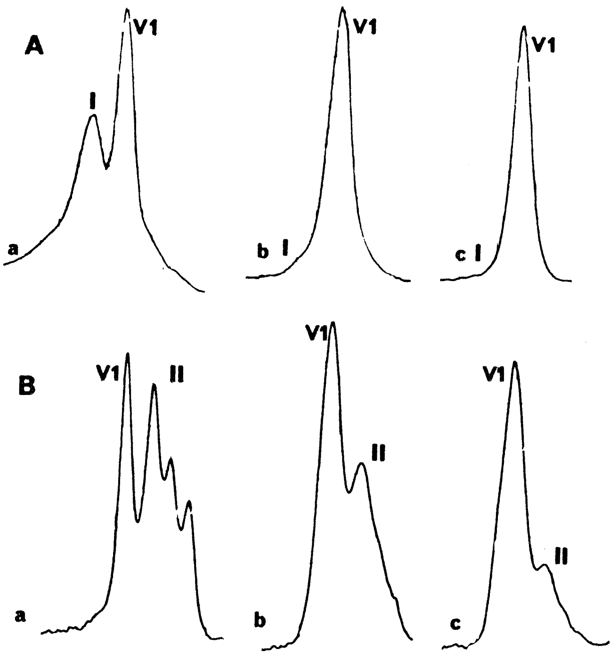


Figure 3 Separation by electrophoresis under non-dissociating conditions of rabbit myosin isoforms and determination of their Ca^{2+}-ATPase activity and pH lability. Densometric scans. a: coelectrophoresis of soleus type I myosin isoform and of masseter (anterior deep and middle masseter) cardiac V1 type myosin. b: electrophoresis of masseter cardiac V1 type myosin isoform and type II myosin isoforms. a: protein staining; b: Ca^{2+}-ATPase activity, at pH 8.8; c: Ca^{2+}-ATPase activity, at pH 8.8, after preincubation at pH 4.3.

This result was confirmed by ATPase assays performed on muscle cross-sections. At pH 9.4, all fibres of the retractor mandibulae displayed ATPase activity, while about half of them only remained active after acid preincubation (Fig. 4). The same results were obtained with the masseter (not shown). Since the fibres containing type II myosin are alkaline-stable but acid-labile, one may deduce that the fibres containing the cardiac myosin are, on the other hand, both alkaline- and acid-stable.

Analysis of the cardiac isoform subunits.
The light chains. Two-dimensional gel electrophoresis (Fig. 5).

The wole adult masseter contained a mixture of light chains. Their positions on the gel indicated that these were of the slow- and fast-type, 1S and 2S, and 1F, 2F, and F3, respectively [28, 3]. The analysis of the anterior deep and middle masseter, which we had shown by electrophoresis under non-dissociating conditions, to contain mainly the cardiac myosin isoform, was greatly enriched in light chains of the slow-type, suggesting that the masseter cardiac myosin contained slow-type light chains. This was confirmed by the observation that the cardiac isoform, purified by electrophoresis under non-dissociating conditions, contained the slow-type light chains exclusively.

As rabbit soleus and ventricles contain the same light chains [13], while rabbit ventricles and auricles contain distinct light chains [22], comigration experiments with soleus, ventricle, and auricle light chains were performed; the results further demonstrated that the light chains of the cardiac-type myosin present in the masseter comigrated with the 1V (or 1S) and 2V (or 2S) light chains of the ventricle (or soleus) and not with the 1A and 2A light chains of the auricle.

The heavy chains. SDS-glycerol gel electrophoresis and immunological reactivity.

The whole adult masseter contained a mixture of at least two types of heavy chain (Fig. 6A), the slower migrating heavy chain corresponding to type II heavy chains and the faster one migrating at the level of the α heavy chain present in the heart auricle (Fig. 6B).

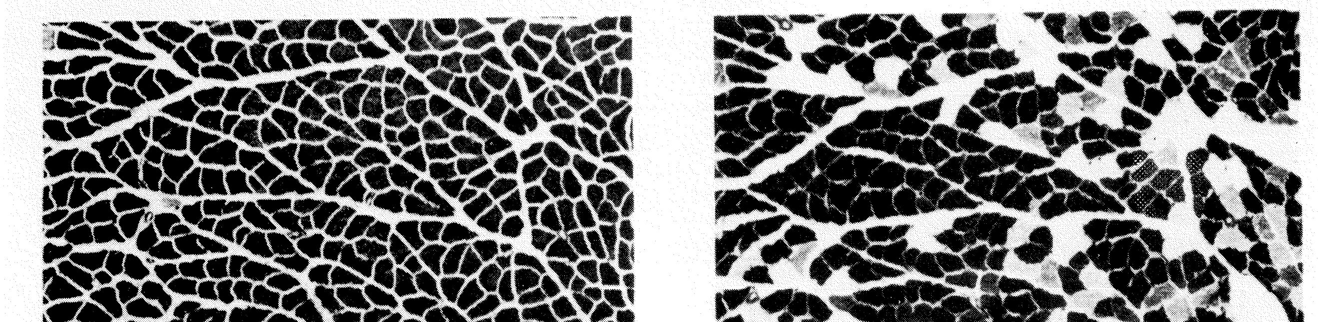


Figure 4 Cross-sections of adult rabbit retractor mandibulae. Myofibrillar ATPase activity at pH 9.4, without (a) and after preincubation (b) at pH 4.3. Bar: 100 µm.

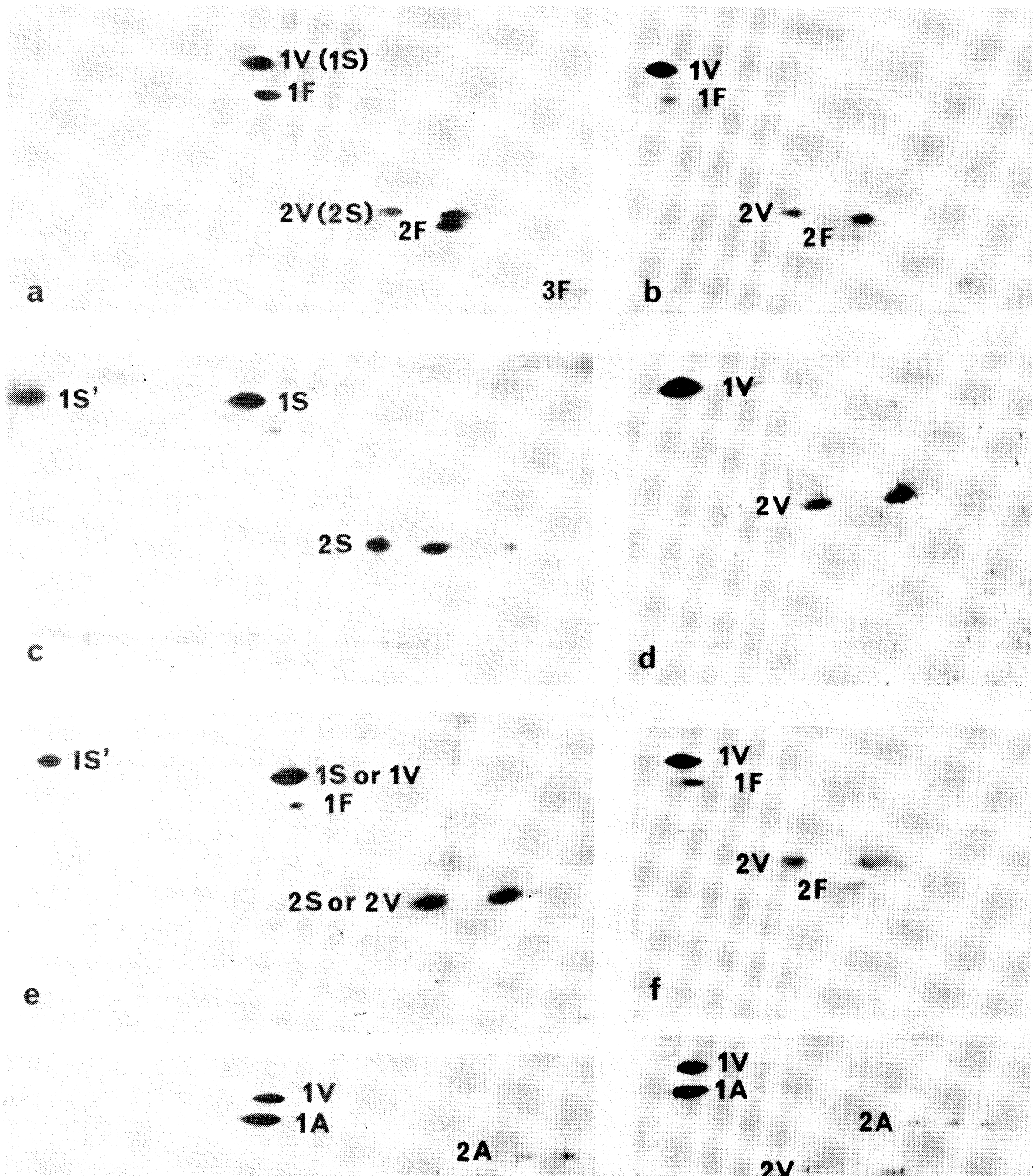


Figure 5 Analysis by two-dimensional gel electrophoresis of the myosin light chains of various adult rabbit muscles. a: masseter; b: anterior deep and middle masseter; c: soleus; d: masseter cardiac V1 myosin (pure V1 myosin was obtained by electrophoresis under non-dissociating conditions - see Fig. 1); e: soleus + anterior deep and middle masseter; f: cardiac ventricles + anterior deep and middle masseter; g: cardiac ventricles + auricles; h: cardiac auricles + anterior deep and middle masseter. 1S, 1S', and 2S: slow-type light chains; 1V and 2V: ventricular light chains (1S = 1V and 2S = 2V); 1F, 2F, and 3F: fast-type light chains; 1A and 2A: auricular light chains.

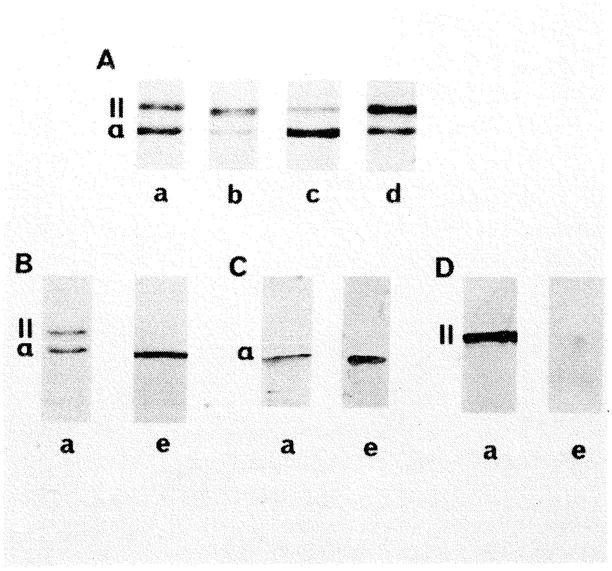


Figure 6 SDS-glycerol gel electrophoresis and immunoblotting of myosin heavy chains. A: Myosin heavy chains in the masseter (a), and in different portions of the masseter (b: posterior deep masseter; c: anterior deep and middle masseter; d: superficial masseter (see Fig. 1)). B - D: Myosin heavy chains in the masseter (a) and in the cardiac auricles (e). B: Protein staining. C: Immunoblotting with an anti-α cardiac myosin heavy chain antibody (249-5A4). D: Immunoblotting with an anti-fast myosin antibody (Sigma). II and α designate the II-type and the cardiac α-type myosin heavy chains, respectively.

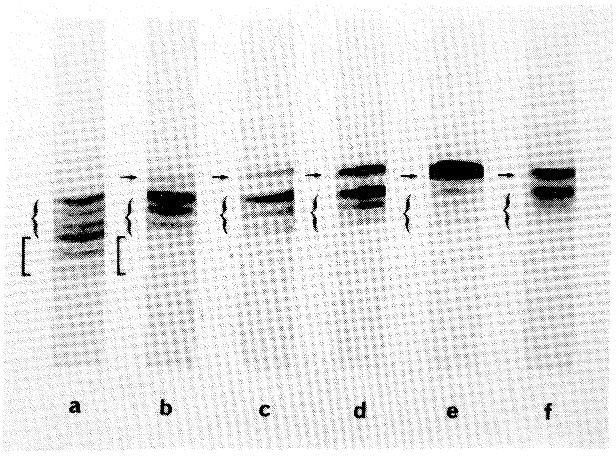


Figure 7 Electrophoresis under non-dissociating conditions of the myosin isoforms present in the masseter during rabbit development. a: 12 days; b: 21 days; c: 25 days; d: 35 days; e: 80 days; f: adult. The symbols designate the different isoforms. ---> ventricular isoform V1, { type II isoforms; [neonatal isoforms.

Various portions of the masseter were shown to contain different proportions of the two heavy chain types (Fig. 6A); as demonstrated by electrophoresis under non-dissociating conditions (see Fig. 1), these portions contained various proportions of the cardiac isoform. Comparison of the results obtained by the two electrophoresis techniques indicated that the heavy chain of the masseter cardiac isoform was of the α-type.

The α-type of the masseter cardiac isoform heavy chain was further confirmed by the fact that its heavy chain cross-reacted with an anti-α myosin heavy chain antibody, whereas it did not react with an anti-fast myosin antibody (Fig. 6C and D). The latter reacted only with the fast-type heavy chains present in the masseter (Fig. 6D).

Developmental regulation of the cardiac myosin isoform

The myosin isoforms present in the masseter between birth and one year of age were analyzed quantitatively by the study of either the native myosins (Fig. 7), or the heavy chains (not shown). Both studies showed that the adult type II myosin isoforms progressively replaced the neonatal isoforms, starting from birth onwards, while the amount of the cardiac isoform became significant starting from the third week postnatal. The proportion of the cardiac isoform in the masseter increased until around three months, when it represented almost 80% of the total myosin isoforms, and then decreased to a relatively stable value of about 50% (Fig. 8).

In good agreement with these results, analysis of light chains showed that the proportion of the ventricular or slow-type light chains increased gradually during the first month postnatal (Fig. 9). At that time, hardly any β and γ actins could be observed (Fig. 9).

A parallel study performed in the retractor mandibulae showed that in this muscle the timing of the appearance and disappareance of the V1 myosin isoform was similar to that in the masseter (Fig. 10).

Neural regulation of the cardiac myosin isoform

When the retractor mandibulae was denervated at 8-day postnatal, the synthesis of the cardiac isoform from the third

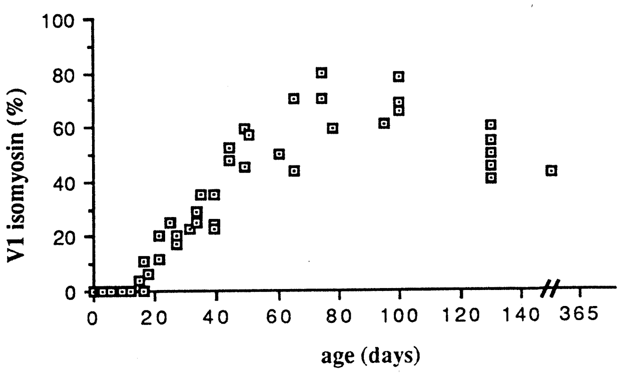


Figure 8 Variation with the age of rabbit of the proportion of the cardiac V1 myosin present in the masseter.

week onward was inhibited, as shown by electrophoresis (Figs. 10 to 12). The inhibition of the cardiac isoform was also observed by cytochemistry, which revealed a dramatic decrease of the acid-stable fibres (Fig. 13).

For comparison, the triceps sural was also denervated. This procedure resulted in the inhibition of the synthesis of type II heavy chains in the gastrocnemius medialis, while the slow heavy chain in both this muscle and in the soleus was not significantly affected (Fig. 12).

When the retractor mandibulae was denervated at 1-month postnatal, the proportion of cardiac isomyosin remained at the level which it had reached at this age (Figs. 10 and 11). Denervation of the retractor mandibulae always induced the resynthesis of neonatal type myosins (Fig. 11).

Discussion

Bredman *et al.* [4] have recently shown by cytoimmunochemistry that rabbit cranial muscles, including the masseter and temporalis, expressed a "cardiac specific" myosin heavy chain. Using biochemical criteria, we confirm in the present study that the mastication muscles of the rabbit contain an α-type cardiac heavy chain and we demonstrate that it is associated to ventricular-type light chains. These results strongly suggest that the cardiac myosin, which is present in high proportion in these muscles, is similar or identical to one of the myosin isoforms present in the heart ventricle, the V1 isoform.

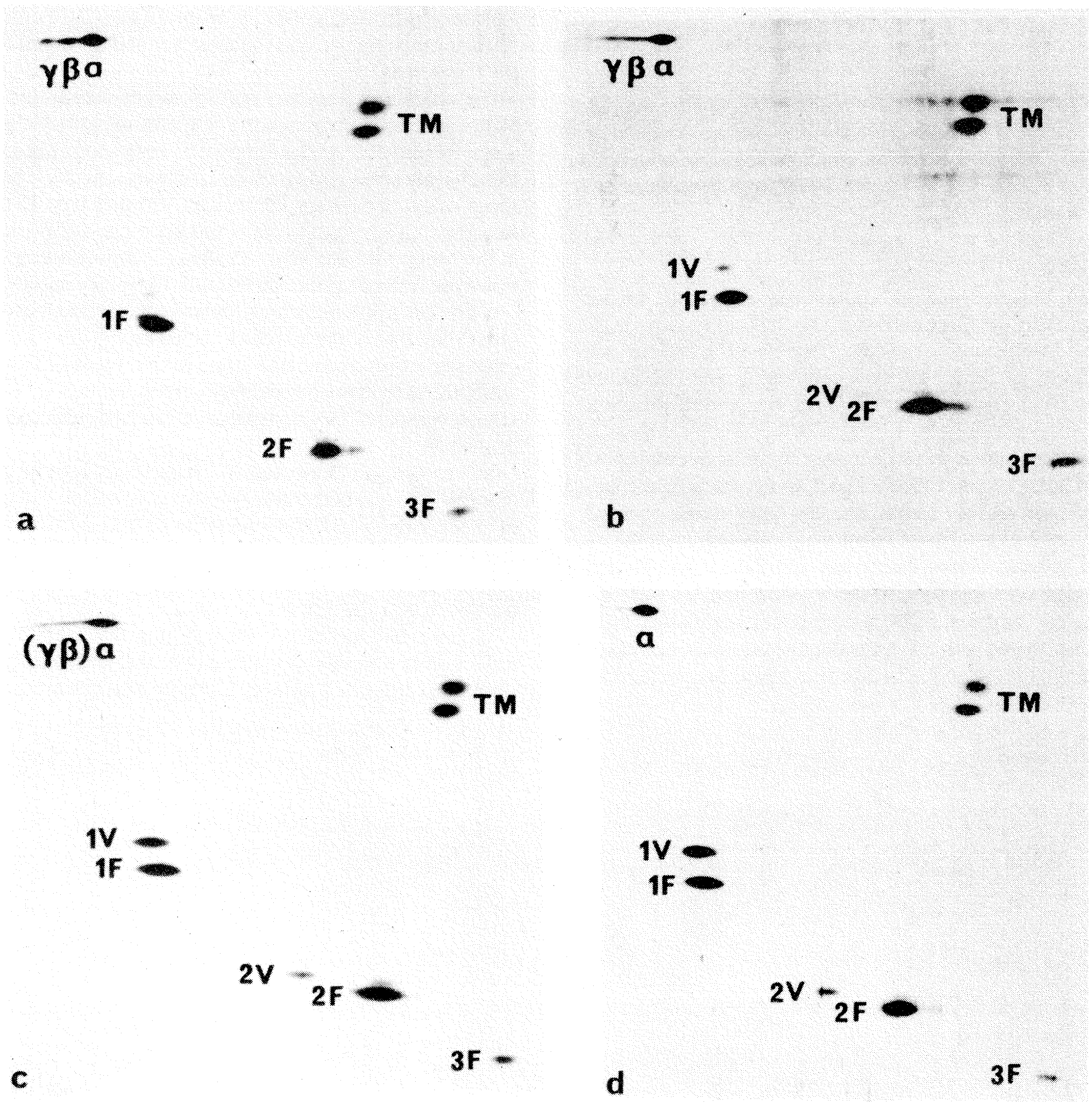
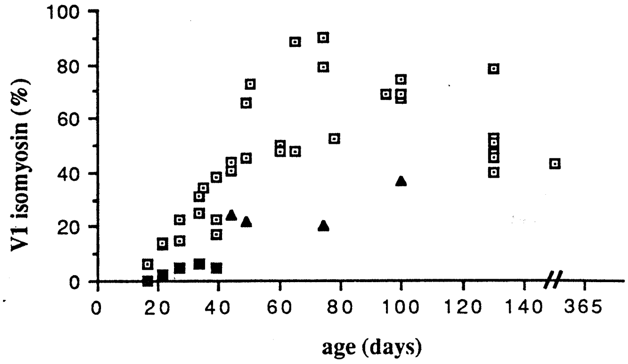


Figure 9 Analysis by two-dimensional gel electrophoresis of myosin light chains, as well as tropomyosin and actin isoforms, present in the masseter during postnatal development of the rabbit. a: 1 day; b: 15 days; c: 21 days; d: 31 days. 1V and 2V: ventricular light chains, and 1F, 2F, and 3F: fast-type light chains. TM: tropomyosin; α, β, γ: actin isoforms.



Fiigure 10. Variation with age of rabbit of the proportion of the cardiac V1 myosin present in the retractor mandibulae. □ *control muscle;* ■ *muscle which wose denervated at 8 days;* ▲ *muscle which wose denervated at 1 month.*

Myosin isoform nomenclature

Skeletal muscles generally contain more than one myosin isoform. There are three main classes of myosin isoforms in the limb and trunk muscles, the type I, the type IIa, and the type IIb isoforms, present in the slow-contracting type I fibres and fast-contracting type IIa and IIb fibres, respectively [reviewed in 23].

The cardiac muscle is also heterogeneous and contains two types of heavy chains, called α and β, two types of light chains 1, the 1V and 1A light chains, and two types of light chains 2, the 2V and 2A light chains. In the ventricles, one finds the $\alpha\alpha$, $\alpha\beta$, and $\beta\beta$ heavy chains, associated with the 1V and 2V light chains, and corresponding to the V1, V2, and V3 isoforms, respectively. In the auricles, one finds the $\alpha\alpha$ and $\beta\beta$ heavy chains, associated with the 1A and 2A light chains, and corresponding to the A1 and A2 isoforms respectively [reviewed in 8]. In the adult rabbit, the ventricles contain V3 and auricles contain A1 [19].

Characterization of a V1 myosin isoform in the adult rabbit masticatory muscles

Masticatory muscles of the rabbit were shown by electrophoresis of myosin in its native form to contain a mixture of type I isoform, type II isoforms, and an isoform not characteristic of any skeletal muscle myosin isoforms so far described.

Type I myosin isoform was present in fact in only trace amounts in the temporalis, the retractor mandibulae, and the masseter; in the masseter, it was largely confined to the posterior deep portion of the muscle.

Type II myosin isoforms - a mixture of IIa and IIb isoforms - accounted for about 50% of the total myosin isoforms in the retractor mandibulae and the masseter and for about 80% in the temporalis.

The unidentified myosin isoform, which constituted about 50% of the total myosin in the retractor mandibulae and the masseter and 20% in the temporalis, corresponded to an isoform having an electrophoretic mobility intermediate between those of the type I (or ventricular V3) and type II myosin isoforms. This myosin isoform had been previously observed in the rabbit masseter and tentatively identified as a peculiar slow-type (or type I) myosin [20, 14]. In fact, we showed:

- that its electrophoretic mobility was comparable, but slightly lower than that of the auricle A1 isoform,
- that it cross-reacted with an anti-α cardiac myosin heavy chain antibody, both in the native and denatured states,
- that it contained 1V (identical to 1S) and 2V (identical to 2S) light chains,
- that its Ca^{2+}-ATPase activity on gels was high at pH 8.8 (contrarily to that of the slow-type myosin), and not inactivated by incubation at pH 4.3 (contrarily to that of the fast-type myosins),
- that its actin-activated ATPase activity on muscle sections was both acid- and alkali-stable.

All these characteristics strongly suggest that rabbit masticatory muscles contain a myosin isoform, that is identical or very close to a cardiac V1 isoform, made up of α heavy chains and

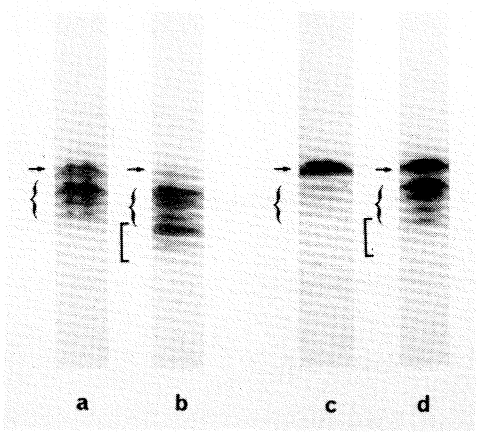


Figure 11 Electrophoresis under non-dissociating conditions of the myosin isoforms present in the retractor mandibulae of 33- (a and b) and 100-day-old (c and d) rabbits. a and c: controls; b and d: muscles denervated at 8 days and 1 month, respectively. The symbols designate the different isoforms. ---> ventricular isoform V1; { type II isoforms; [neonatal isoforms

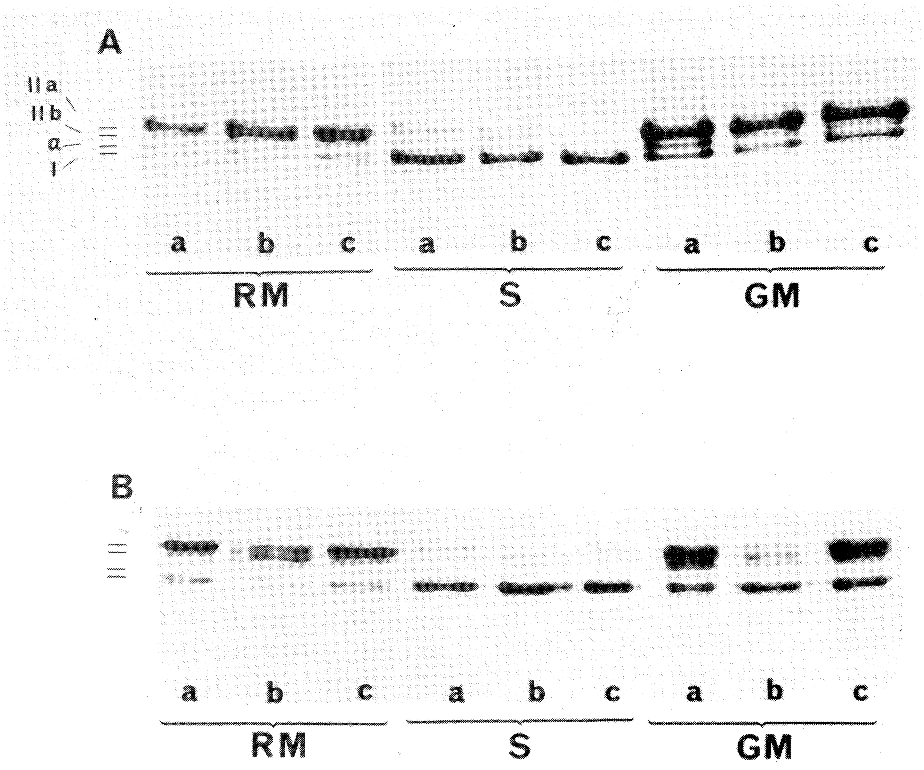


Figure 12 SDS-glycerol gel electrophoresis of myosin heavy chains of retractor mandibulae (RM), soleus (S), and gastrocnemius medialis (GM). A: 21- and B: 39-day-old rabbits. a: control muscles; b: muscles denervated at 8 days; c: controlateral muscles. IIa, IIb, α, and I designate the two II-type, the cardiac α-type, and the I-type myosin heavy chains, respectively.

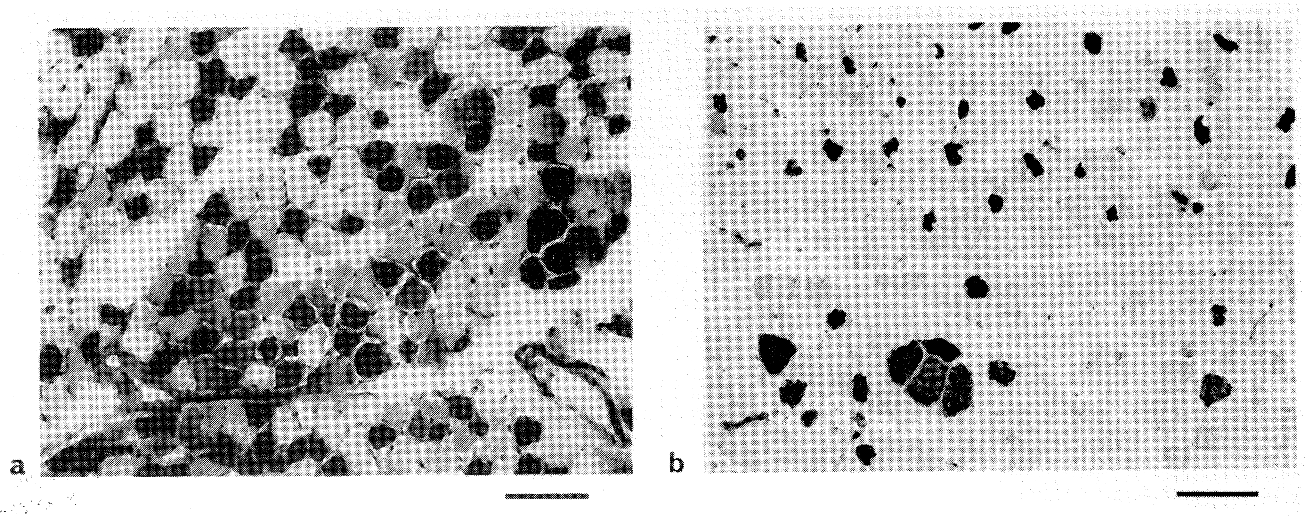


Figure 13 Cross-section s of 43-day-old rabbit retractor mandibulae. Myofibrillar ATPase activity at pH 9.4, after preincubation at pH 4.3 Bar: 100 μm. a: control muscle; b: muscle denervated at 8 days

of ventricular (or slow) light chains. The presence of a high proportion (about half of the total myosins) of V1 in the adult rabbit masseter explains earlier contradictory results, where it was found that this muscle contains many slow-type light chains, but few slow-type fibres [20]. It is in excellent accordance with the finding that numerous rabbit masseter fibres are both acid- and alkali-stable [29, 24, 5] and especially, as first shown by Bredman *et al.* [4], that they cross-react with an anti-α myosin antibody.

Developmental variation of the V1 isoform in the retractor mandibulae and masseter

It has been repeatedly observed that neonatal myosin isoforms, which, as their name indicates, are normally expressed in mammalian skeletal muscles during the perinatal life, are progressively inhibited and replaced by adult type-II isoforms. The time at which this replacement occurs varies with the muscle [10] and the animal species [11 bis]. In the rat, the adult myosins appear later in the masseter than in any of the 11 other muscles that we investigated and "neonatal" myosins continue to be synthesized in the adult muscle [11]. This persistence of the synthesis of neonatal myosins in the masseter has also been observed in the human masseter [31]. In the rabbit masseter, on the other hand, three weeks after birth, neonatal myosins were no longer significantly expressed.

We observed in this study that the V1 isoform started to appear after birth, but became significant only at the beginning of the third week postnatal, when almost all neonatal myosins had disappeared. Its synthesis was thus delayed in comparison to that of fast-type II myosins, which indicates that the two types of myosin isoforms display different developmental and epigenetic regulations. The proportion of V1 reached a maximum value of about 80% at around three months and then decreased to an apparently stable value of 50 ± 10%.

The proportion of V1 varied with the age of the rabbit in the same way in the retractor mandibulae and the masseter.

The proportion of V1 also increases and then decreases in the cardiac ventricle as a function of the age of the rabbit [19]. No physiological significance for this change has been proposed so far, but it is clear that it could be related to the changes in thyroid hormone concentration during development [reviewed in 34].

Effect of the denervation of the retractor mandibulae on the synthesis of V1

"Skeletal-type" myosin isoforms are known to be differently regulated by innervation. Denervation of slow and fast muscles of newborn rats inhibits the synthesis of the slow-type myosin, but apparently does not affect that of fast-type myosins [27, 6]. How the cardiac-type myosins are controlled by innervation is, on the other hand, not known, since denervation of the heart raises problems.

It was thus of interest to investigate the effect of denervation on the synthesis of V1 in the rabbit masticatory muscles and to compare it to the effect of denervation on the synthesis of the slow and fast type myosins. The retractor mandibulae, as well as the soleus and gastrocnemius medialis muscles, were denervated on 8 day-old rabbits; the soleus contains mainly the slow-type myosins and the gastrocnemius medialis a mixture of slow- and fast-type myosins. No significant effect on V1 was observed two weeks after the operation, since at the age of three weeks, the amount of V1 in the retractor mandibulae is in any case very small. On the other hand, the type IIb myosin was apparently already more affected than the type I and IIa myosins. One month after the operation, it became obvious that the V1 myosin was inhibited by the denervation, and that all type II myosins were also inhibited, while the slow type I myosin appeared scarcely affected.

The observation that, in the rabbit, contrarily to what has been established in the rat, slow myosin was not inhibited by denervation, while fast myosins were inhibited, fits with cytochemical results obtained by Bacou and Vigneron [2].

It is also interesting that denervation of the retractor mandibulae induced the reexpression of "neonatal" type myosins in the adult rabbit. As stated above, these myosins are not normally synthesized in the adult muscle: thus, the masticatory muscles exhibit a particular aptitude to remodelling. This characteristic was previously demonstrated in the rat masseter, in which neonatal myosins are reexpressed after hyperthyroidism treatment had been interrupted [9].

Masticatory muscles differ from the trunk and limb muscles by their embryologic origin - branchiomeric instead of mesodermal - and by their innervation - by cranial instead of spinal nerves [25]. In a previous work on the masseter of small rodents, we had evoked these features to possibly account for the persistence of neonatal-type myosin isoforms in the adult rat and mouse muscle [11]. One might now ask if the presence of a large amount of a cardiac-type V1 myosin isoform in the masticatory muscles of the rabbit could be related to the particular embryologic origin and innervation of the muscles. It is probably not the case, since we found that the V1 myosin isoform was transiently expressed in the rabbit diaphragm.

Different arguments also induced Soussi-Yanicostas *et al.* [32] and Bredman *et al.* [4] to reject this kind of explanation; these authors suggested that either special functional needs or/and a specific environment might play roles in inducing the expression of atypical myosin isoforms in some skeletal muscles. The high plasticity of the masticatory muscles is reminiscent of that of the cardiac muscle [reviewed in 34]. It may thus not be totally fortuitous that these muscles in the rabbit contain a V1 cardiac-type myosin.

It is known that the so-called embryonic light chain is identical to the atrial light chain 1A, and that the slow myosin isoform is composed of the same light (1S = 1V, and 2S = 2V) and heavy (I = β) chains as the ventricular V3 isoform [reviewed in 34]. The α-type heavy chain has been described in several rabbit cranial muscles [4] and we identified in the present work that this heavy chain is associated to 1V and 2V light chains and thus constitutes a V1 myosin isoform. More studies may show progressively that cardiac-type myosin isoforms are expressed in other skeletal muscles. Qualifying these isoforms as cardiac may then rely only on a historical basis.

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References

[1] Ansorge W: Fast visualisation of protein bands by impregnation in potassium permanganate and silver nitrate, in Stathakos D (ed): *Electrophoresis 82*. Berlin, New York, de Gruyter, 1983, pp 235-242.

[2] Bacou F, Vigneron P: Polymorphisme de l'acétylcholinestérase et de la myosine au cours du développement des muscles rapide et lent dénervés chez le lapin nouveau-né. *Reprod. Nutr. Develop.* 1988; 28: 757-768.

[3] Biral D, Damiani E, Volpe P, Salviati G, Margreth A: Polymorphism of myosin light chains. An electrophoretic and immunological study of rabbit skeletal-muscle myosin. *Biochem. J.* 1982; 203: 529-540.

[4] Bredman JJ, Weijs WA, Moorman AFM: Expression of "cardiac specific" myosin heavy chain in rabbit cranial muscles, in Maréchal G, Carraro U (eds): *Muscle and Motility*. Andover, Hampshire, Intercept, 1990; pp 329-335.

[5] Bredman JJ, Weijs WA, Moorman AFM, Brugman P: Histochemical and functional fibre typing of the rabbit masseter muscle. *J. Anat.* 1990; 168: 31-47.

[6] Butler-Browne GS, Bugaisky LB, Cuénoud S, Schwartz K, Whalen RG: Denervation of newborn rat muscles does not block the appearance of adult fast myosin heavy chain. *Nature (London)* 1982; 299: 830-833.

[7] Carraro U, Catani C: A sensitive SDS-Page method separating myosin heavy chain isoforms of rat skeletal muscles reveals the heterogeneous nature of the embryonic myosin. *Biochem. Biophys. Res. Commun.* 1983; 116: 793-802.

[8] Cummins P: Cardiac myosin isozymes. *ISI Atlas Science* 1988; 169-174.

[9] d'Albis A, Chanoine C, Janmot C, Mira JC, Couteaux R: Muscle-specific response to thyroid hormone of myosin isoform transitions during rat postnatal development. *Eur. J. Biochem.* 1990; 193: 155-161.

[10] d'Albis A, Couteaux R, Janmot C, Roulet A: Specific programs of myosin expression in the postnatal development of rat muscles. *Eur. J. Biochem.* 1989; 183: 583-590.

[11] d'Albis A, Janmot C, Béchet JJ: Comparison of myosins from the masseter muscle of adult rat, mouse and guinea-pig. Persistence of neonatal-type isoforms in the murine muscle. *Eur. J. Biochem.* 1986; 156: 291-296.

[11 bis] d'Albis A, Janmot C, Couteaux R: Species- and muscle type-dependence of perinatal isomyosin transitions. *Int. J. Dev. Biol.*. 1991; 35: 1-4.

[12] d'Albis A, Pantaloni C, Béchet JJ: An electrophoretic study of native myosin isoenzymes and of their subunit content. *Eur. J. Biochem.* 1979; 99: 261-272.

[13] Dalla Libera L: A comparative study of atrial and ventricular myosin light subunits from different species. *Comp. Biochem. Physiol.* 1986; 83B: 751-755.

[14] Gregory P, Low RB, Stirewalt WS: Fractional synthesis rates *in vivo* of skeletal-muscle myosin isoenzymes. *Biochem. J.* 1987; 245: 133-137.

[15] Guth L, Samaha FJ: Qualitative differences between actomyosin ATPase of slow and fast mammalian muscle. *Exp. Neurol.* 1969; 25: 138-152.

[16] Hoh JFY, Hughes S, Chow C, Hale PT, Fitzsimons RB: Immunocytochemical and electrophoretic analyses of changes in myosin gene expression in cat posterior temporalis muscle during postnatal development. *J. Muscle Res. Cell Motility* 1988; 9: 48-58.

[17] Hoh JFY, McGrath PA, White RI: Electrophoretic analysis of multiple forms of myosin in fast-twitch and slow-twitch muscles of the chick. *Biochem. J.* 1976; 157: 87-95.

[18] Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London)* 1970; 680-685.

[19] Lompré AM, Mercadier JJ, Wisnewsky C, Bouveret P, Pantaloni C, d'Albis A, Schwartz K: Species and age-dependent changes in the relative amounts of cardiac myosin isoenzymes in mammals. *Dev. Biol.* 1981; 84: 286-290.

[20] Mabuchi K, Pinter K, Mabuchi MS, Sreter F, Gergely J: Characterization of rabbit masseter muscle fibers. *Muscle Nerve* 1984; 7: 431-438.

[21] O'Farrell PH: High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 1975; 250: 4007-4021.

[22] Oh-Ishi M, Hirabayashi T: Comparison of protein constituents between atria and ventricles from various vertebrates by two-dimensional gel electrophoresis. *Comp. Biochem. Physiol.* 1989; 92B: 609-617.

[23] Pette D, Staron RS: Cellular and molecular diversities of mammalian skeletal muscle fibers. *Rev. Physiol. Biochem. Pharmacol.* 1990; 116: 1-76.

[24] Rowlerson A, Mascarello F, Barker D, Saed H: Muscle-spindle distribution in relation to the fibre-type composition of masseter in mammals. *J. Anat.* 1988; 161: 37-60.

[25] Rowlerson A, Mascarello F, Veggetti A, Carpene E: The fibre-type composition of the first branchial arch muscles in Carnivora and Primates. *J. Muscle Res. Cell Motil.* 1983; 4: 443-472.

[26] Rowlerson A, Pope B, Murray J, Whalen RB, Weeds AG: A novel myosin present in cat jaw-closing muscles. *J. Muscle Res. Cell Motil.* 1981; 2: 415-438.

[27] Rubinstein NA, Kelly AM: Myogenic and neurogenic

contributions to the development of fast and slow twitch muscles in rat. *Dev. Biol.* 1978; 62: 473-485.

[28] Schachat FH, Bronson DD, McDonald OB: Two kinds of slow skeletal muscle fibers which differ in their myosin light chain complements. *FEBS Lett.* 1980; 122: 80-82.

[29] Schiaffino S: Histochemical enzyme profile of the masseter muscle in different mammalian species. *Anat. Rec.* 1974; 180: 53-62.

[30] Shelton GD, Cardinet GH, Bandman E: Expression of fiber type specific proteins during ontogeny of canine temporalis muscle. *Muscle Nerve* 1988; 11: 124-132.

[31] Soussi-Yanicostas N, Barbet JP, Laurent-Winter C, Barton P, Butler-Browne GS: Transition of myosin isozymes during development of human masseter muscle. Persistence of developmental isoforms during postnatal stage. *Development* 1990; 108: 239-249.

[32] Soussi-Yanicostas N, Breuer EM, Dang DC, Butler-Browne GS, in Maréchal G, Carraro U (eds): *Muscle and Motility.* Andover, Hampshire, Intercept, 1990; pp 63-70.

[33] Suzuki A: A comparative histochemical study of the masseter muscle of the cattle, sheep, swine, dog, guinea pig, and rat. *Histochem.* 1977; 51: 121-131.

[34] Swynghedauw B: Developmental and functional adaptation of contractile proteins in cardiac and skeletal muscles. *Physiol. Rev.* 1986; 66: 710-771.

[35] Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci.* USA 1979; 76: 4350-4354.

[36] Weijs WA, Brugman P, Klok EM: The growth of the skull and jaw muscles of the rabbit and its functional consequences in the New Zealand rabbit (*Oryctolagus cuniculus*). *J. Morph.* 1987; 194: 143-161.

[37] Young OA, Davey CL: Electrophoretic analysis of proteins from single bovine muscle fibres. *Biochem. J.* 1981; 19: 317-327.