Cardiac-Type Myosin and Fibers in Rabbit Masticatory Muscle after Cardiotoxin Injury

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Abstract

New myogenesis following cardiotoxin injury of the rabbit retractor mandibulae (RM) muscle was studied by determination of myosin subunits and by fiber typing. Cardiac-type myosin and fibers reappeared after the injury. As in normal muscles, their proportions were different in females and males, suggesting that satellite cells have a genetic program and hormonal regulation similar to those of myoblasts.

Key words: regeneration, myogenesis, cardiac myosin, satellite cell, rabbit masticatory muscle, sexual hormone.

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The retractor mandibulae (RM) and other masticatory and cranial muscles of rabbit contain a V1 cardiac-type myosin, made up of α myosin heavy chains (MHC) and slow or ventricular myosin light chains (MLC) [8, 2, 3]. Whereas this myosin isoform is present in the heart at birth and then progressively completely disappears, it first appears in RM in significant amounts only 3 weeks after birth; its proportion then increases up to 50 to 60% of all RM myosin in male and female rabbits by age 2 months. Thereafter, its production continues in females but decreases in males; castration of males reinduces its production [5]. The inhibition of cardiac myosin in males may thus be associated with the onset of sexual hormone production, at about two months in the male rabbit.

To investigate the mechanism of the late appearance of V1 myosin in the RM of rabbit, we studied its production in muscle whose fibers had been destroyed by cardiotoxin and which undergoes a new myogenesis *in vivo*, *via* the surviving satellite cells. The myosin subunits and the fiber types of the regenerating muscles were characterized at various times after cardiotoxin treatment, which was applied to 8- and 30-day-old rabbits.

Materials and Methods

Muscle injury

Experiments were performed on 8- and 30-day-old New-Zealand rabbits provided by CEGAV (13 male and 9 female 8-day-old and 10 male and 11 female 30-day-old rabbits). The RM was exposed by incision of the overlying skin and injected with a cardiotoxin purified from *Naja mossambica mossambica* venom (10 µM in 0.9% NaCl); the muscle was then bathed in the toxin solution for 5 min, after which it was washed with Ringer solution [6]. The muscle was carefully dissected at various times after the injury, between the age of 30 and 180 days, and immediately frozen in liquid nitrogen for biochemical studies and in isopentane cooled in liquid nitrogen for cytochemical studies. Its properties were compared to those of non-injured contralateral and control muscles.

Myosin preparation

Myosin was extracted with a high ionic strength solvent as described in [7].

SDS-glycerol gel electrophoresis of myosin heavy chains

MHC were separated in 8% polyacrylamide slab gels, in the presence of 0.4% SDS and 30% glycerol [16], at 70 V for 28 h at 8°C [11].

Two-dimensional gel electrophoresis of myosin light chains

MLC were separated by the technique described by O'-Farrell [14], using a narrow pH range (4.5 to 5.5) for high sensitivity, as described in [7].

The relative amounts of the different MHC and MLC isoforms, stained with Coomassie Blue, were measured with a densitometer equipped with an integrator.

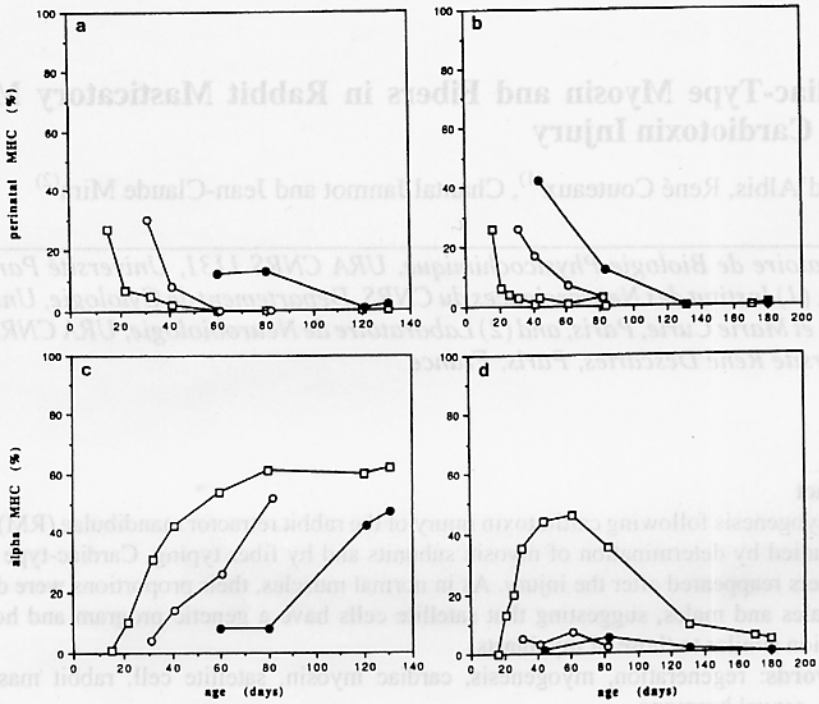

Figure 1. Variation with age of the proportion of perinatal (a and b) and α cardiac (c and d) MHC in the female (a and c) and in the male (b and d) RM rabbits. □ Contralateral muscles. 8-day-old cardiotoxin-injured muscles. | 30-day-old cardiotoxin-injured muscles.

Cytochemical methods

Cryostat cross-sections (10 µm) were incubated at pH 9.4 to measure muscle fiber Ca^{2+}-activated adenosine triphosphatase after preincubation at pH 4.3 or treated with cardiac myosin isoform monoclonal antibody (Biosys). Photographs were taken through a microscope equipped for fluorescence [7].

Results

The amounts of the various MHC in the rabbit RM were determined by SDS gel electrophoresis (Fig. 2). At 15 day-postnatal, the contralateral RM of female and male rabbits contained about 20% perinatal- and 80% 2a fast-type MHC. At 2 months, it contained about 50% of both α-cardiac and 2a fast-type MHC. At 4 months, there was a difference between the sexes, the female RM containing about 60% and the male RM less than 10% α-cardiac MHC. These variations with age are shown in Fig. 1.

The proportions of perinatal and α-cardiac MHC in cardiotoxin-treated RM of female and male rabbits were determined in the same way and are reported in Fig. 1. In the injured muscles, perinatal MHC was transiently produced; this production was greater in males than in females. Production of α-cardiac MHC was small in males. In females it was delayed in comparison with its production in contralateral (or control - not shown) muscles; however the proportion of α-MHC increased with time to a level eventually similar to that in the contralateral muscles.

The MLC content was determined in the RM of control and cardiotoxin-treated RM of female and male 120-day-old rabbits (Fig. 3). Slow- or ventricular-type MLC, normally associated with α-cardiac MHC in V1-type myosin, were present in high proportion in the female contralateral and cardiotoxin-treated muscles, only slightly present in the male contralateral muscle, and absent in the male cardiotoxin-treated muscle.

Fibers were typed both by an ATPase myofibrillar assay and by immunocytochemistry. Sections of 30-day-old cardiotoxin-treated muscles are shown in Fig. 4. One hundred and twenty days after the injury, about half the fibers in the female muscle were enzymatically active after preincubation at pH 4.3 and reactive with an antibody directed against cardiac myosin, indicating that they were of the cardiac-type; only few fibers were of the cardiac-type in the male muscle.

Discussion

Regeneration myogenesis in the adult is believed to recapitulate development myogenesis in the perinatal state [for review, 15]. Most regeneration experiments have been performed in rat and some in mouse and in cat, but we are unaware of any in rabbit. In rat, production of adult-type myosins is more precocious in regeneration myogenesis than in development myogenesis, due to the activating effect of thyroid hormone [6, 9].

The results reported here indicate in contrast that in regeneration myogenesis of the rabbit RM perinatal myosin persisted longer and cardiac myosin production was much delayed in comparison with that in normal postnatal muscle development; it was in fact much delayed in females and almost completely inhibited in males. This delay can be related to our previous observation that thyroid hormones do not up-regulate the expression of cardiac myosin when expressed in RM [5]. The difference observed between the two sexes may on the other hand be

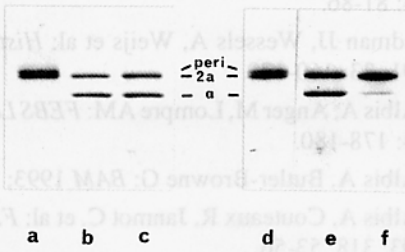

Figure 2. SDS polyacrylamide gel electrophoresis of RM MHC. a, b, c: MHC of 15-, 60- and 120-day-old female rabbits. d, e, f: MHC of 15-, 60- and 120-day-old male rabbits. peri, 2a, and α designate the perinatal, fast-type 2a, and cardiac-type MHC, respectively.

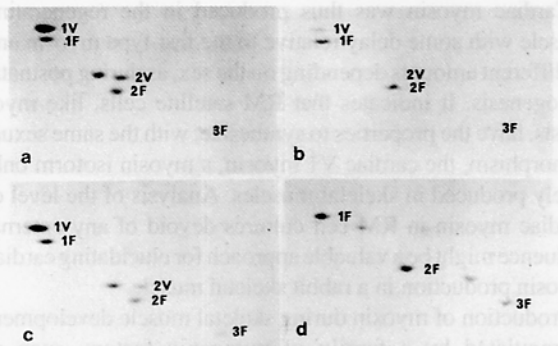

Figure 3. Two-dimensional gel electrophoresis of 120-day-old rabbit RM MLC. a: Contralateral female rabbit RM. b: Contralateral male rabbit RM. c: 30-day-old cardiotoxin-injured female rabbit RM. d: 30-day-old cardiotoxin-injured male rabbit RM. 1F, 2F and 3F: fast-type MLC; 1V and 2V: slow or ventricular MLC.

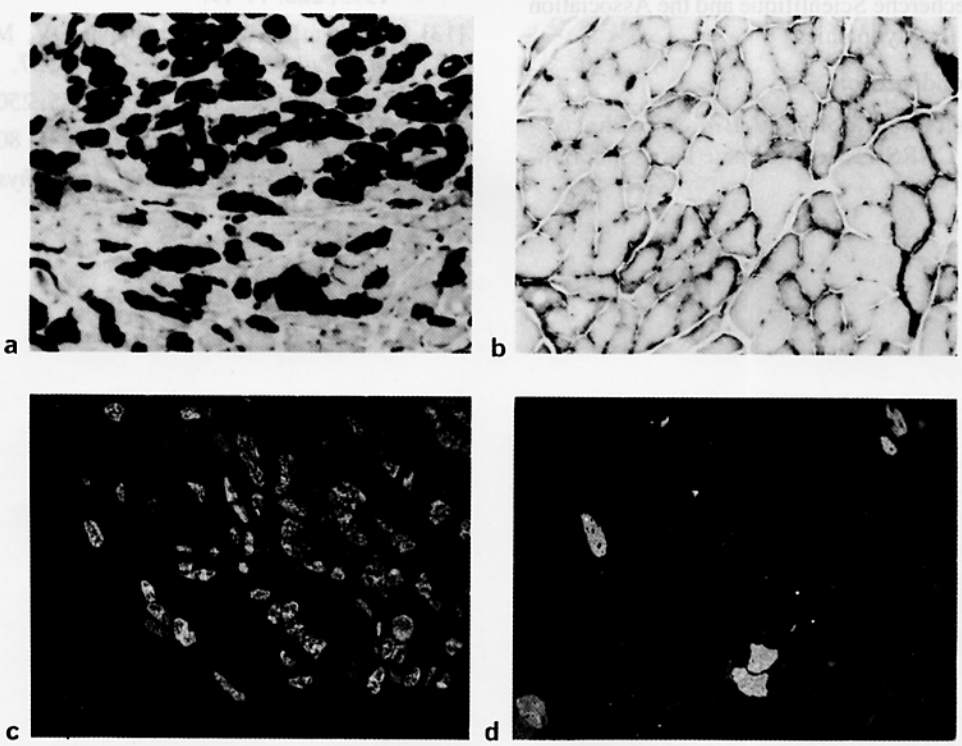

Figure 4. Cross-sections in 30-day-old cardiotoxin-injured RM of 120-day-old rabbit. a and c: female rabbit RM. b and d: male rabbit RM. a and b: Myofibrillar ATPase activity at pH 9.4 after preincubation at pH 4.3. c and d: Labelling with a cardiac myosin antibody. Bar: 100 μm.

attributed to the repressive effect of male hormones on the production of cardiac myosin in the RM [5].

Cardiac myosin was thus produced in the regenerating muscle with some delay relative to the fast-type myosin and in different amounts depending on the sex, as during postnatal myogenesis. It indicates that RM satellite cells, like myoblasts, have the properties to synthesize, with the same sexual dimorphism, the cardiac V1 myosin, a myosin isoform only rarely produced in skeletal muscles. Analysis of the level of cardiac myosin in RM cell cultures devoid of any external influence might be a valuable approach for elucidating cardiac myosin production in a rabbit skeletal muscle.

Production of myosin during skeletal muscle development is regulated by a family of myogenic factors, such as myogenin. The expression of myogenin in regenerating skeletal muscle has been recently demonstrated [12]. No such myogenic factors are present in heart muscle, but it has been suggested that GATA-4 protein may be a specific activating factor of the heart genetic program [10]; two GATA motifs have been found in the cardiac myosin gene promotor [13]. It will thus be interesting to examine whether the GATA sequence is present in RM - and in other masticatory muscles of the rabbit containing cardiac myosin - and if present to compare GATA expression in the male and female muscles during postnatal development.

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