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The same myosin isoforms are found in the female and male sexually dimorphic levator ani muscle of the rat, but their postnatal transitions are not synchronous

Anne d'Albis[1], René Couteaux[2], Chantal Janmot[1] and Jean-Claude Mira[2,3]

[1]*Laboratoire de Biologie Physicochimique, URA CNRS 1131, Université Paris-Sud, Orsay, France,* [2]*Institut des Neurosciences du CNRS, Département de Cytologie, Université Pierre et Marie Curie, Paris, France* and [3]*Laboratoire de Neurobiologie, Université René Descartes, Paris, France*

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The levator ani of the female adult rat is greatly atrophied in comparison to the same muscle in males. In the present study, the female levator ani was, nevertheless, found to contain type IIb myosin isoforms similar to those contained in the male muscle. These adult type isoforms were, however, synthesized later in the female than in the male levator ani: the half-transition times of the myosin transition curve were 20 days postnatal in the male and 35 days postnatal in the female. The transition curves for castrated and uncastrated male rats were the same. Thus, the presence of male gonadal hormones apparently did not affect the myosin transition.

Myosin isoform; Postnatal transition; Levator ani; Gonadal hormone; Castration

1. INTRODUCTION

A number of muscles involved in vertebrate reproduction are particularly sensitive to androgens and display sexual dimorphism. These muscles include the levator ani, which is well developed in male rats but, as shown by Tobin and Joubert [1], very atrophied in females. In fact, prior to their study, it had been thought that the levator ani in the female rat underwent complete involution during the postnatal period, since it is not supplied with testosterone [2].

Among the known sexually dimorphic muscles, the guinea-pig temporalis and the *Xenopus laevis* larynx muscles have been shown to contain muscle fibers of different types in males and females [3,4]. Lyons et al. [3] established that in the temporalis male and female muscle fibers had different myosin isoform contents.

Considering that the levator ani develops differently in male and female rats, it was of interest to examine (i) whether the adult female muscle contained the same adult myosin isoforms as those found in the adult male muscle [5]; (ii) whether the postnatal transition from embryonic and neonatal to adult myosin isoforms occurred at the same time in both females and males; (iii) whether the myosin transition in the male was under the influence of male gonadal hormones.

Correspondence address: A. d'Albis, Laboratoire de Biologie Physicochimique, Bât. 433, Université Paris-Sud, 91405 Orsay, France

Abbreviations: EDL, extensor digitorum longus; LC1, LC2 and LC3, myosin light chains; SDS, sodium dodecyl sulfate

2. EXPERIMENTAL

2.1. *Animals and muscles*

The levator ani was carefully dissected from 3-month-old Sprague-Dawley and Wistar female and male rats, as well as from 16-75-day-old females and 3-35-day-old males. The EDL muscle of the adult rat was also dissected for comparison.

2.2. *Castration*

It was performed on newborn male rats.

2.3. *Myosin preparation*

Myosin was extracted with a high ionic strength buffer [5].

2.4. *Gel electrophoresis of native myosin isoforms*

Electrophoresis under nondissociating conditions was performed in a buffer consisting of 20 mM sodium pyrophosphate (pH 8.5), 10% glycerol, 0.01% 2-mercaptoethanol and 2 mM $MgCl_2$ [5]. The proportions of embryonic and neonatal type isoforms and of adult isoforms were determined by densitometry.

2.5. *Glycerol-SDS gel electrophoresis of myosin heavy chains*

This was performed, according to [6], on 6% acrylamide gels in the presence of 35% glycerol.

2.6. *Two-dimensional gel electrophoresis of myosin light chains*

Isoelectric focusing was performed on cylindrical gels between pH 4.5 and 5.5. These gels were then applied to 15% acrylamide plate gels and run in the presence of SDS [5].

3. RESULTS

3.1. *Myosin isoforms in the adult female levator ani*

We observed that the adult female levator ani contained 3 myosin isoforms, which were resolved by electrophoresis under nondissociating conditions. These 3

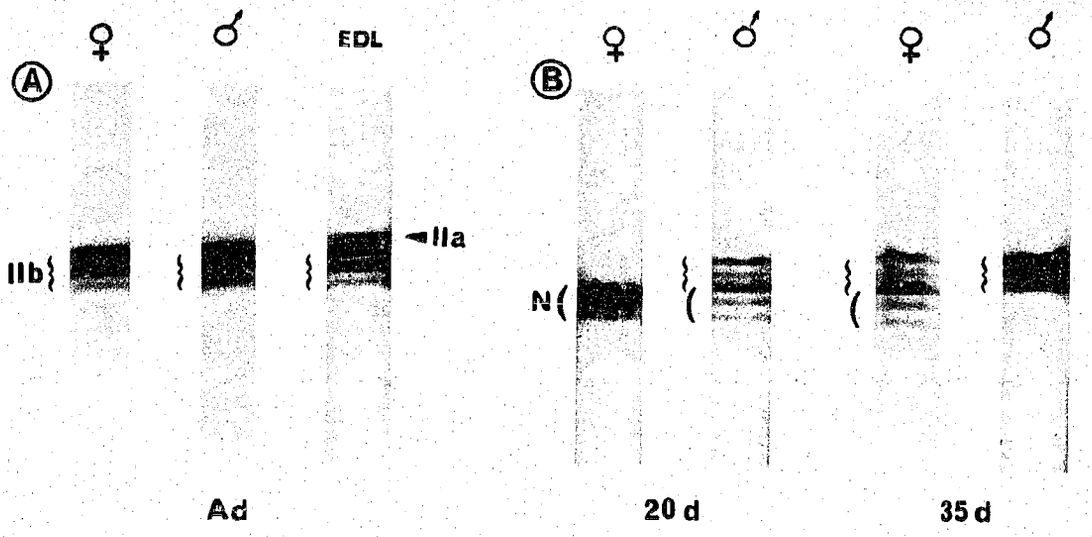

Fig. 1. Separation of native myosin isoforms by electrophoresis under non-dissociating conditions. (A) Adult (Ad) rat muscles. Female (♀) and male (♂) levator ani and EDL. (B) Postnatal female (♀) and male (♂) levator ani, at 20 and 35 days. IIa (◄) and IIb ⟨) correspond to the IIa- and IIb-type adult myosin isoforms and "N(" corresponds to the neonatal myosin isoforms.

isoforms comigrated with those previously characterized in the male muscle [5]. Their relative proportions varied from one adult muscle to the other, both in male and female rats. These isoforms were typical of type IIb fibers; in contrast, the EDL muscle, which contains both type IIa and type IIb fibers, displayed an additional myosin band of lower electrophoretic mobility (Fig. 1A).

A study of levator ani myosin heavy and light chains revealed the presence of a single type IIb heavy chain (Fig. 2A), and of 2 alkali light chains, LC1 and LC3, together with the LC2 light chain (Fig. 2B). These results indicate that the 3 myosin isoforms present in the levator ani corresponded to 3 alkali light chain isoforms, the LC1 and the LC3 homodimers and the LC1 + LC3 heterodimer, respectively.

We have thus shown that, in contrast to other sexual dimorphic muscles [3,4], the adult levator ani contains the same myosin isoforms in females as in males and that these isoforms are of the IIb type.

3.2. *Postnatal transition to adult myosin isoforms*

We demonstrated recently [5] that the adult type myosins were not synthesized at the same time in the different muscles of the same animal. In the rat muscles we have investigated, the transition occurred earliest in the diaphragm and last in the masseter, with half-transition times of 7 and 23 days postnatally, respectively. The transition in the levator ani was also late, with a half-transition time of 20 days.

In the previous study [5], only the levator ani of male rats was examined. Although the female muscle is very

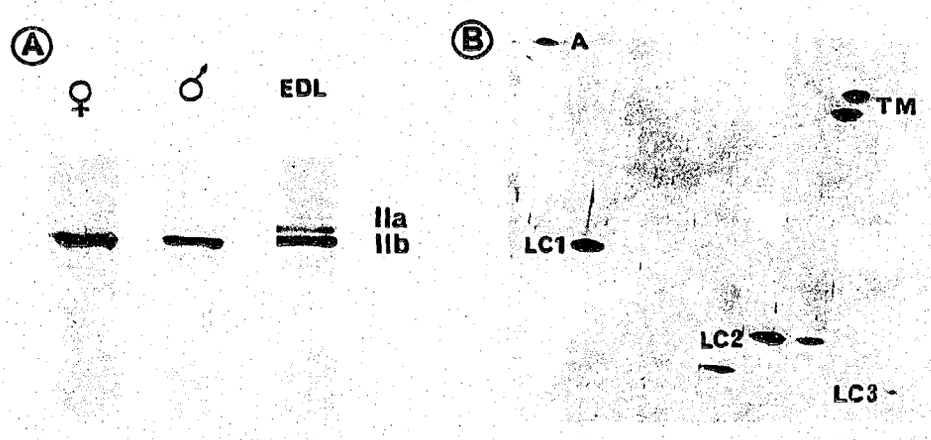

Fig. 2. Separation of adult myosin heavy and light chains by electrophoresis under dissociating conditions. (A) Electrophoresis of the heavy chains in the presence of glycerol-SDS. Female (♀) and male (♂) levator ani and EDL. (B) Two-dimensional separation of the LC1, LC2, and LC3 light chains of the levator ani myosin. Actin (A) and the 2 spots of tropomyosin (TM) are also present on the same portion of the gel.

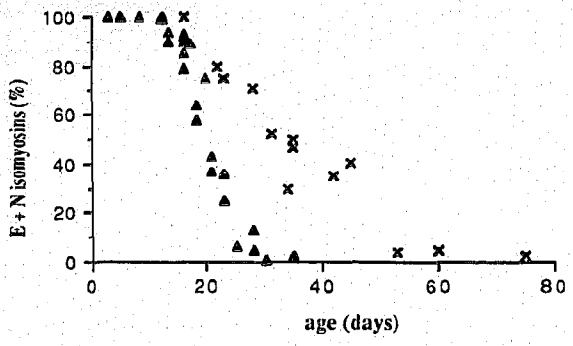

Fig. 3. Postnatal myosin transition from the embryonic (E) and neonatal (N) myosin isoforms to the adult isoforms in the female (×) and male (▲) levator ani.

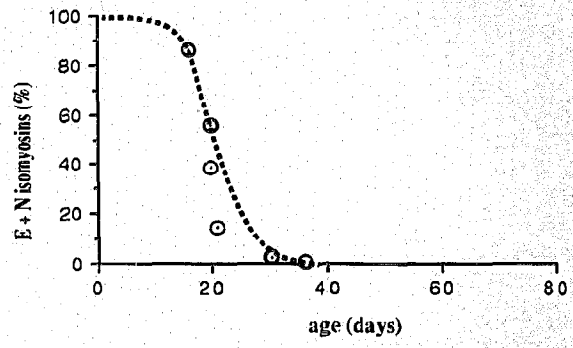

Fig. 4. Postnatal myosin transition from the embryonic (E) and neonatal (N) myosin isoforms to the adult isoforms in the male levator ani. The dotted curve corresponds to the experimental points for control rats (see Fig. 3) and the symbols (⊙) correspond to the values obtained for rats which have been castrated at birth.

small, it was possible to dissect it by the 3rd week postnatally and to measure quantitatively the adult myosins it contained at several ages between 16 and 75 days postnatally.

Fig. 1B displays the myosin isoforms present in the levator ani of 20- and 35-day-old female and male rats. As in the case of the male muscle [5], the neonatal type isoforms in the female muscle could be distinguished from the adult type isoforms by their higher electrophoretic mobilities under nondissociating conditions. At 20 days, the female levator ani exhibited mainly neonatal isoforms, while in males of the same age adult isoforms represented about 50% of the total. At 35 days, adult isoforms in the female represented about 50% of the total, whereas at this age only adult isoforms were present in the male muscle.

The delay in the transition to adult isoforms in the female levator ani is best illustrated in Fig. 3. The half-transition in females occurs at about the age of 35 days, whereas it occurs at 20 days in the male muscle.

3.3. *Effect of castration on the myosin transition in the male levator ani*

The castration of newborn male rats provoked the progressive atrophy of the levator ani, without inducing any change in the developmental appearance of the adult myosin isoforms (Fig. 4).

4. DISCUSSION

In contrast with other sexually dimorphic muscles, which exhibit fibers and myosins of different types in males and females [4,5], the levator ani in adult rats of both sexes was shown in this study to contain the same adult myosin isoforms. On the other hand, there was a marked difference in the developmental regulation of these adult isoforms, since the adult isoforms appeared later in females than in males.

The change in the plasma concentration of gonadal hormones during postnatal development [7] might be the reason for this difference. However, it has been established that the onset of puberty in Wistar and Sprague-Dawley rats occurs at about 27 days postnatally in males and at 34 days postnatally in females [8]. There is thus no clear correlation between the development of the reproductive system which takes place during this period and the myosin transition curves. This lack of correlation was confirmed by the castration experiments we performed on male rats: castration was not accompanied by any significant change in the myosin transition curve of the levator ani.

The fact that neuromuscular synapse elimination occurs late in the levator ani [9] cannot be a cause for the delay in myosin transition in this muscle, since synapse elimination appears to be related to the increase in androgen [10], which we showed in this work to have no influence on the myosin transition.

Since neither the change in the type of innervation nor the presence of gonadal hormones seems to explain the difference in the chronology of the postnatal regulation of the adult myosin isoforms between male and female levator ani, we suggest either that the muscle is differently genetically programmed in the 2 sexes, or that during the postnatal period its development remains under the influence of hormones other than the gonadal androgens.

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