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# Opposite regulations by androgenic and thyroid hormones of V1 myosin expression in the two types of rabbit striated muscle: skeletal and cardiac

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The finding that V1 cardiac myosin is expressed in masticatory skeletal muscles of the rabbit [1] provided a unique opportunity for comparing the hormonal regulation of V1 in skeletal and cardiac muscles. Thyroid hormones had no significant effect on the postnatal expression of V1 in masticatory muscles, but increased this expression in cardiac ventricles. In contrast, androgenic hormones reduced V1 expression in masticatory muscles, but did not affect it significantly in cardiac ventricles. Modulation of V1 gene transcription in striated muscle is thus shown here to depend both on the target muscle and on the hormone.

Cardiac V1 myosin; Thyroid hormone; Androgenic hormone; Masticatory muscle; Development; Rabbit muscle

## 1. INTRODUCTION

Skeletal muscles and cardiac muscle constitute the two different types of striated muscle. Although skeletal muscle cells are elongated and multinuclear, whereas cardiac muscle cells have a single nucleus, the contractile apparatus is much the same in both types of striated muscle, with thick myosin and thin actin filaments aligned transversely. Similarly, the myosin content is partly different and partly the same in the two types of striated muscle. In mammals, skeletal muscles contain two developmental myosin isoforms and four adult isoforms, the slow myosin and three types of fast myosin; the cardiac ventricular muscle contains three myosin isoforms, called V1, V2, and V3. V3 is the same isoform as the slow myosin isoform of skeletal muscles. All these isoforms are expressed in muscles in different relative amounts, depending on muscle type, species and age (for review, see [2]).

In the rabbit, the cardiac ventricular muscle contains a mixture of the V1, V2, and V3 myosin isoforms until about 1 month after birth; thereafter, the V3 isoform predominates [3,4]. In skeletal muscles, developmental myosin isoforms are expressed until just before or just after birth, depending on the muscle; they are then replaced by adult isoforms [5]. In the rabbit masseter, the skeletal muscle studied here, adult myosin isoforms start to appear during the week after birth and are of the fast type. As we showed in a previous study [1], rabbit masseter contains, in addition, a myosin isoform, which is either identical or very similar to the V1 cardiac type myosin isoform; this is also true of other masticatory muscles, for instance retractor mandibulae. This V1 isoform appears in the rabbit masticatory muscles during the third week after birth, and at 3 months may constitute as much as 80% of the total amount of myosin [1].

The exceptional presence of V1 myosin in skeletal muscles provided an excellent opportunity for comparing the regulation of its expression in the two categories of striated muscle. We therefore studied the effects of androgenic and thyroid hormones on the expression of V1 in rabbit masticatory muscles and cardiac ventricle.

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A preliminary report of these data was presented at the XXI European Muscle Congress, Bielefeld (Germany) in September 1992.

## 2. EXPERIMENTAL

### 2.1. *Animals and experimentation*

New Zealand rabbits were provided by CEGAV. Castration was performed on 8-day-old male rabbits. Experimental hyperthyroidism was induced in male and female rabbits by intraperitoneal injections of 2 μg 3,5,3'-triiodothyronine ($T_3$) per 10 g body weight every other day, starting on day 8 after birth.

### 2.2. *Muscle sampling and myosin extraction*

The masseter and retractor mandibulae, and the cardiac left ventricle were dissected from control and experimental rabbits aged from 1 day to 6 months and immediately frozen in liquid nitrogen. Myosin was extracted as in [6].

### 2.3. *Electrophoretic analysis of native myosin isoforms*

Electrophoresis was performed as first described by d'Albis and Gratzer [7], with a few modifications [1]. 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.

## 3. RESULTS

### 3.1. *Gel separation of the V1 myosin isoform*

Examples of gels are given in Fig. 1. Fig. 1a shows the electrophoretic patterns of the myosin in the 4-month-old rabbit masseter, which contained the V1 isoform and 3 fast-type isoforms; the control female masseter, as well as the castrated male masseter, contained 68% V1, but the control male masseter only contained 33%. Fig. 1b shows the electrophoretic patterns of the myosin in 5-week-old rabbit ventricles, containing a mixture of V3, V2, and V1 isoforms; the control ventricle contained 8% V1, but the ventricle from a hyperthyroid rabbit contained 67% V1.

### 3.2. *Effects of androgenic hormones*

We found that in the rabbit masseter and retractor mandibulae, the amount of V1 relative to the total amount of myosin (i.e. to the sum of V1 and fast-type myosins) varied with age (Fig. 2a and b): V1 appeared in males and females during the third week after birth, exhibited parallel increases in both sexes during the first two months of life, and then went on increasing in the females but started decreasing in the males.

We suspected that this down-regulation of V1 expression in the males was due to the action of androgenic hormones. Accordingly, castration of the males one week after birth induced expression of V1 in the castrated males similar to that in the females (Fig. 2a and b).

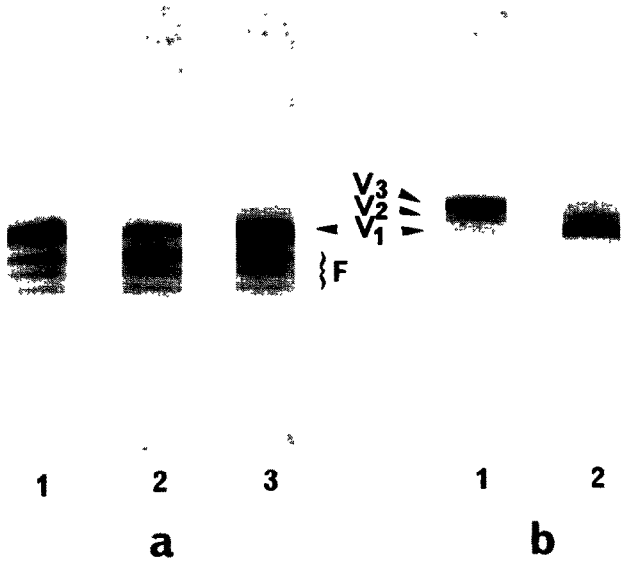


Fig. 1. (a) Myosin isoform content in the masseter of 4-month-old rabbits: 1, control female masseter; 2, control male masseter; 3, castrated male masseter. (b) Myosin isoform content in the left ventricle of 5-week-old rabbits: 1, control ventricle; 2, ventricle of a hyperthyroid rabbit; F, fast isoforms; V1, V2, V3, cardiac isoforms.

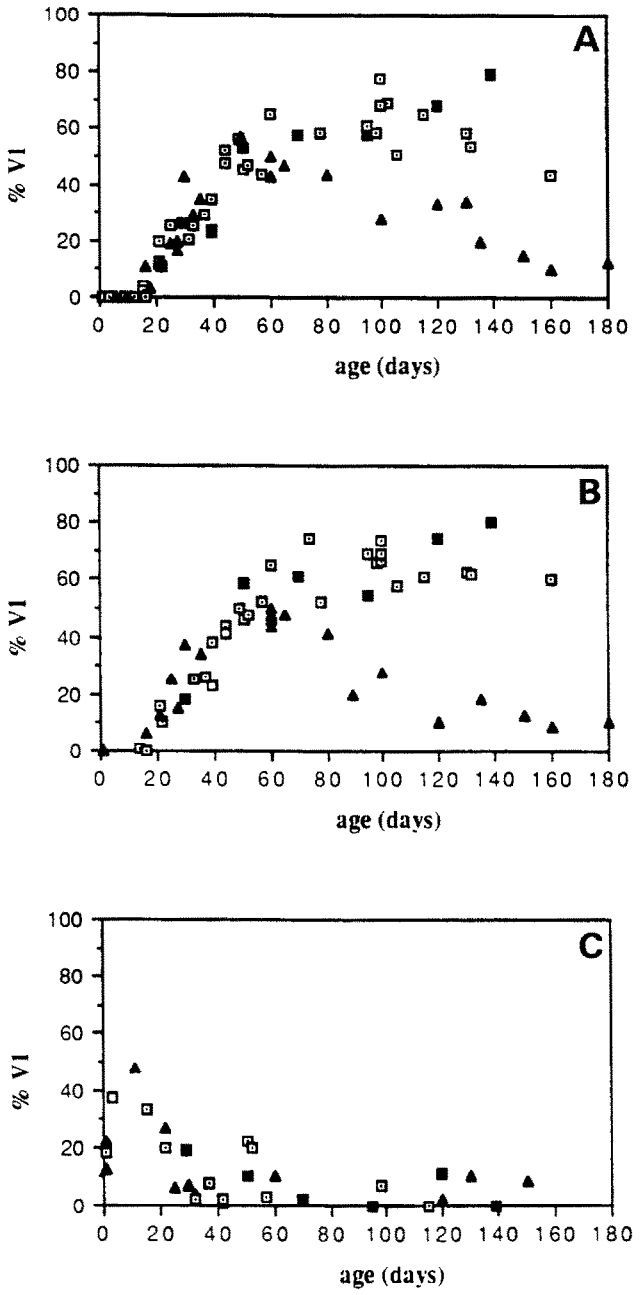


Fig. 2. Variation with age in the proportion of V1 myosin, (a) in masseter, (b) in retractor mandibulae, (c) in left ventricle. □, control females; ▲, control males; ■, castrated males.

Concomitantly, we examined the postnatal expression of V1 in the cardiac ventricle of the same animals. The amount of V1 relative to the total amount of myosin (i.e. to the sum of V1, V2, and V3) first increased and then decreased steadily, dropping to about 10% from the age of 1 month onwards (Fig. 2c). Unlike the masticatory muscles, male and female ventricles exhibited the same variations in V1 levels, which were not significantly modified by castration of the males.

### 3.3. *Effects of thyroid hormones*

The effect of inducing experimental hyperthyroidism by $T_3$ treatment on V1 expression in the masseter and retractor mandibulae muscles was examined up to the age of 2 months, i.e. during the period when the variations of V1 with age were the same in males and females (Fig. 3a and b). $T_3$ treatment had no significant effect on the expression of V1.

Concomitantly, the effect of hyperthyroidism on V1 expression was tested in the ventricle of the same animals, and was found to cause a large increase in the relative amount of V1 (Fig. 3c).

## 4. DISCUSSION

In mammals, the masseter muscle is highly specialized and exhibits unusual species variability; its myosin composition varies from the slow type exclusively in ruminants, to exclusively fast types in guinea-pig (for reviews, see [8,9]). In the rabbit, the masseter, as well as another masticatory muscle, the retractor mandibulae, contains, in addition to fast myosins, large amounts of a myosin similar to the V1 myosin, made up of α type heavy chains and slow type light chains. The presence of a myosin heavy chain of the α cardiac type was observed by immunocytochemical methods in cranial muscles of the rabbit [10,11]; concomitantly with the latter investigations, we determined, by several methods, that this heavy chain was associated with slow-type light chains, and therefore belonged to a myosin of the ventricular V1 type [1].

By contrast with the well-known up-regulation by thyroid hormone of V1 myosin expression in the cardiac ventricle of the rabbit [12–14], which was confirmed in this work, we observed that, in the masseter and retractor mandibulae, hyperthyroidism did not change V1 expression during postnatal development. One could argue that skeletal muscles display less plasticity than the cardiac muscle, and that thyroid treatment may take longer to become effective in the masseter and retractor mandibulae than in cardiac ventricle. However, this explanation does not seem plausible, because our experiments lasted for two months, and because we previously showed that the action of thyroid hormones on rat skeletal muscles was fast [15]. In addition, the postnatal appearance of V1 in the masticatory muscles did not coincide with the postnatal rise in the level of endogenous thyroid hormone [16]. The present results therefore point to the conclusion that V1 myosin isoform is regulated differently by thyroid hormone, depending on the muscle, skeletal or cardiac, in which it is expressed; a similar observation had been made for other myosin isoforms between different skeletal muscles [17] and between ventricle and atria [18].

A second peculiar feature of the expression of V1 in the masticatory muscles was its sex-dependence. Up to the age of 2 months, the curves showing the variations

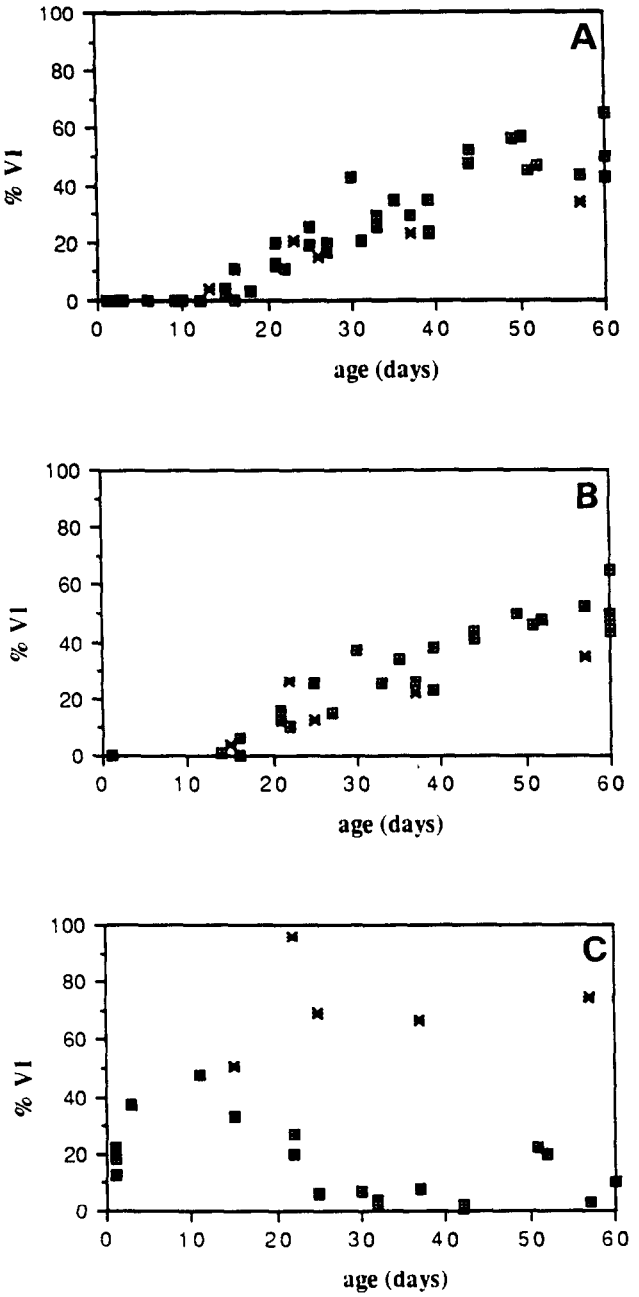


Fig. 3. Variation with age in the proportion of V1 myosin, (a) in masseter, (b) in retractor mandibulae, (c) in left ventricle. ⊞, control females and males; ×, hyperthyroid females and males.

in the expression of V1 were the same in males and females; from 2 months onwards, the curves diverged: the proportion of V1 remained fairly stable in the females at 60 to 80%, but dropped steadily in the males, down to 10%. This is in good agreement with the unpublished finding by Mascarello and Rowlerson, reported in a review by Rowlerson [8]: 'sex-related differences in the fibre type composition of jaw-closer muscles do not occur in juveniles but only in adults'.

The divergence between males and females may be

connected with the onset of spermatogenesis at about 2 months, when the androgenic hormone concentration in males is maximal [19]. The present castration experiments performed on males confirmed that androgenic hormones inhibit the expression of V1, as in the masseter and retractor mandibulae they induced increased synthesis of V1, whose proportion rose to the level found in the females. Consequently, the rabbit masseter and retractor mandibulae belong to the small family of sexually dimorphic muscles, of which the best known are the rat levator ani [20] and the guinea-pig temporalis [21,22]; in this connection, we previously showed that the rat masseter also displayed some sexual dimorphism [6].

In contrast with the inhibition by androgenic hormones of V1 expression in the masticatory muscles, we did not find that male castration significantly affected V1 expression in the ventricle. To our knowledge, this is the first such investigation to be performed on the rabbit. Unlike what we found in this species, castration is known to repress V1 synthesis in the ventricle of male rats [23–25]. This difference between the effects of castration on the rabbit and rat may be due to species, but it may also be only apparent: thus, in the young rat, in which the myosin isoform V1 is predominant, the repressive effect of castration is easily observed; on the other hand, in the young rabbit, V1 myosin disappears rapidly, so that the repressive effect of castration might pass unnoticed. In any case, whether the effect of castration on V1 expression in rabbit ventricle is negative or non significant, it differs from its effect on V1 expression in the masticatory muscles, where it is positive.

The present results therefore point to the conclusion that the same myosin isoform is regulated in opposite ways by thyroid and androgenic hormones depending on the hormone and target muscle, even though the receptors of both hormones are nuclear and belong to the same protein family [26]. Thyroid and androgenic hormones are known to generally modulate myosin isoform synthesis at the transcriptional level, and therefore directly reflect DNA regulation (for review, see [27]). Considering that thyroid hormone receptors exist in several forms, which may elicit different physiological responses, the mechanisms of positive or negative effects of the hormone-receptor complex on gene transcription remain now to be further elucidated.

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