Effect of Testosterone and Thyroid Hormone on the Expression of Myosin in the Sexually Dimorphic Levator Ani Muscle of Rat*

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During postnatal development, the myosin transition from embryonic and neonatal isoforms to adult isoforms has been shown to occur with half-transition times of about 20 and 32 days in the male and female levator ani muscles, respectively. We show that this difference could not be attributed to the testosterone male hormone, since treatment of newborn females by testosterone did not modify the half-transition time. However, treatment of females by thyroid hormone accelerated the myosin transition of the female muscle, which then occurred at almost the same time as the transition of the male muscle. This suggests that the difference between the half-transition times of the male and female levator ani muscles may be largely attributed to different sensitivities of the male and female muscles to thyroid hormone. This is the first example of sexually dimorphic muscle response to thyroid hormone.

The levator ani is a hormone-dependent and sexually dimorphic muscle (Wainman and Shipounoff, 1941; Hayes, 1965; Galavazi and Sausma, 1971). It is present in the rat at birth in both sexes (Cihák et al., 1970); however, whereas the muscle grows steadily in the male, it does not develop in the female and remains atrophied (Tobin and Joubert, 1988). We have nevertheless been able to show that the female adult muscle contains the same type IIB isomyosins as the male adult muscle. However, the chronology of the transition from embryonic and neonatal to these adult isomyosins differs between the sexes; the age at which 50% of the myosin is adult isomyosins is about 20 days for the male and about 32 days for the female (d'Albis et al., 1991).

The mechanisms underlying this sexual disparity are unknown. However, the testosterone male hormone is a possible regulatory factor for this process. We thus examined the effect on the appearance of the adult isomyosins in the levator ani of treating newborn females with testosterone.

A second hormone, the thyroid hormone, which is known to play a major role in regulating the chronology of the appearance of adult isomyosins, was also a possible regulatory factor (reviewed in Bandman (1985), Swinghedauw (1986), and Vigneron et al. (1989)). We thus also examined the effect of experimental hyperthyroidism on the transition from embryonic and neonatal to adult isomyosins.

EXPERIMENTAL PROCEDURES

Animals—Female and male rats (Sprague-Dawley) were each divided into four groups: controls, testosterone-treated rats, thyroid hormone-treated rats, and testosterone- and thyroid hormone-treated rats.

Hormonal Treatment—Testosterone treatment was performed by subcutaneous injection of 0.1 mg of testosterone propionate on both the first and second days after birth (Tobin and Joubert, 1991). Hyperthyroidism was induced by subcutaneous injection of 3.5 mg of 3,5,3'-triiodothyronine/10 g of body mass, on alternate days, from the third day after birth until sacrifice (d'Albis et al., 1990).

Muscle Sampling, Myosin Preparation, and Electrophoretic Analysis—The levator ani was carefully dissected at various ages, between birth and 2.5 months. The muscle was weighed, and myosin was extracted with a high ionic strength buffer. The isoform composition of myosin was analyzed by electrophoresis under nondissociating conditions.

RESULTS

Effect of Testosterone Treatment on the Isomyosin Transition—As previously observed (Gutmann et al., 1967; Tobin and Joubert, 1991), the treatment of female newborn rats by testosterone induced a large increase in the weight of the levator ani (Fig. 1). However, the transition curves of testosterone-treated female and control female rats were similar. The half-transition time from neonatal to adult isomyosins (Fig. 2) was thus 3 ± 3 days for both groups (Fig. 3), whereas that of control males was 20 ± 1 days (Fig. 4).

Testosterone treatment of male rats was also accompanied by an increase in the weight of the levator ani (not shown) but neither produced any change in the chronology of myosin transition (not shown).

Effect of Hyperthyroidism on the Isomyosin Transition—The treatment of male and female rats by thyroid hormone induced a small but significant decrease in the weight of the levator ani muscle. This was of no technical consequence for the analysis of the muscle myosin isoform content in the male, but resulted in the levator ani in the female being too small for satisfactory analysis. To examine the effect of the thyroid hormone on the myosin transition in the female levator ani, testosterone treatment was required to get enough material for electrophoresis analysis.

In the male, the effects of thyroid hormone alone and of thyroid hormone combined with testosterone were similar and, as previously observed in the case of thyroid hormone alone, induced a small shift in the myosin transition curve. Adult isoforms appeared slightly earlier; the half-time of the
**Hormone Regulation of Myosin**

**Fig. 1.** Weight of the levator ani muscle of female rats plotted against age. X, control females; □, testosterone-treated females.

**Fig. 2.** Myosin isoforms in the levator ani of testosterone-treated female rats at various ages. Analysis was by electrophoresis under nondissociating conditions. a, gels; b, corresponding densitometric scans. II, adult type II isoforms; N, neonatal isoforms.

**Fig. 3.** Postnatal myosin transition from the embryonic (E) and neonatal (N) isoforms to the adult isoforms in the female levator ani muscle. X, control females. □, testosterone-treated females.

**Fig. 4.** Postnatal myosin transition from the embryonic (E) and neonatal (N) isoforms to the adult isoforms in the male and female levator ani muscle. ▲, control males. X, control or testosterone-treated females.

**Fig. 5.** Postnatal myosin transition from the embryonic (E) and neonatal (N) isoforms to the adult isoforms in the male and female levator ani muscle. ■, thyroid hormone-treated or thyroid hormone- and testosterone-treated males. △, thyroid hormone and testosterone-treated females.

**FIG. 3.** Postnatal myosin transition from the embryonic (E) and neonatal (N) isoforms to the adult isoforms in the female levator ani muscle. X, control females. □, testosterone-treated females.

**DISCUSSION**

The myosin isoform transition in the levator ani muscle occurs at different times in the male and female rat. The transition in the male occurs earlier than it does in the female (d’Albis et al., 1991). It thus appears that the myosin transition is differentially regulated in the two sexes.

Epigenetic factors are known to be involved in regulating the expression of myosin, and we therefore examined the possible roles of two hormones, testosterone and thyroid hormone.

As the levator ani is a sexually dimorphic muscle, we first investigated the effect of testosterone on the myosin transition of the female. No significant effect was observed. This result is consistent with studies showing that castration of the male has no effect on the myosin transition (d’Albis et al., 1991).

The possible differential regulatory effect of thyroid hormone on the myosin transition in the male and female levator ani was then investigated.

The endogenous level of plasmatic thyroid hormone is known to increase during postnatal growth of the rat (Gambke et al., 1983; Chizzonite and Zak, 1984; d’Albis et al., 1990). This increase is well correlated with the myosin transition from neonatal isoforms to adult type II isoforms (Gambke et
al., 1983; Butler-Browne et al., 1984; d’Albis et al., 1987). However, we showed that the transition occurs at different times depending on the muscle, the diaphragm being the most precocious and the masseter the latest (d’Albis et al., 1989). Each muscle thus displays a specific response to thyroid hormone, as further confirmed by experimental hyperthyroid and hypothyroid treatments (d’Albis et al., 1990).

No difference between the chronology of the myosin transitions in the male and female rats was observed for any of the muscles we tested, with the exception of two: the masseter (d’Albis et al., 1989) and the levator ani (d’Albis et al., 1991). The difference between the half-transition times of male and female muscles is only about 1 day in the case of the masseter, but it is about 12 days in the case of the levator ani. We therefore examined whether the large difference observed for the transition of the male and female levator ani muscles might be due to different thyroid hormone sensitivities of the male and female muscles.

The transition curve for the levator ani in experimentally induced hyperthyroid males was slightly shifted, and the half-time of transition was about 18 days, as compared with about 20 days in the controls. This confirms previous results (d’Albis et al., 1990) and demonstrates that the endogenous level of thyroid hormone is almost optimal for inducing the myosin transition.

The treatment of female rats by thyroid hormone, on the other hand, induced a large shift in the myosin isoform transition to give a half-time of about 20 days, as compared with about 32 days in the controls. The female transition thus became almost superimposable on the male transition. This result shows that, contrarily to the male, the endogenous level of thyroid hormone in the female is largely suboptimal, although the same as in the male, which suggests that the difference observed between the myosin transitions of the male and female rats can be mainly attributed to different sensitivities of the male and female levator ani muscles to thyroid hormone. This could be due to, for example, different numbers of thyroid hormone receptors in the two sexes.

To our knowledge, there is no other example of a muscle displaying different responses to the thyroid hormone in the male and in the female. This result emphasizes the preponderant role of thyroid hormone in the regulation of the expression of myosin isoforms.

REFERENCES

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