Effect of Hypothyroidism on the Expression and Transitions of Some Developmental Isoforms of Troponin T in Rat Striated Muscles

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Abstract

The effect of thyroid hormones was investigated on the expression of adult and developmental isoforms of troponin T in striated muscle. Hypothyroidism reduced the rate of suppression of foetal and neonatal isoforms of troponin T in developing rat skeletal muscles but showed no effect on the adult muscles. Hypothyroidism did not induce any changes in the pattern of expression of adult and embryonic cardiac troponin T in either the adult or developing cardiac muscle.

Keywords: Hypothyroidism, muscle, troponin T, foetal, neonatal, adult, skeletal, cardiac.

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Our recent immunochemical studies using antibodies to fast troponin T have identified four developmental isoforms of this protein in developing rat skeletal muscles [9]. Two of these called foetal, FF1 and FF2 and the other two called neonatal isoforms, NF1 and NF2 were first detected during the late foetal to early neonatal period and these were generally undetectable in a large majority of the muscles after 1-2 months of age. The developmental isoforms were gradually replaced by a number of adult isoforms of fast troponin T (AF1-AF5). Most adult skeletal muscles expressed different amounts of these five major isoforms. The changes in the troponin T isoforms also occur in the cardiac muscle during development [8,10]. The major isoform detected in the foetal rat cardiac muscle is an embryonic isoform of cardiac troponin T. This is gradually replaced by adult cardiac troponin T during late foetal to neonatal period. The transitions of developmental isoforms in both skeletal and cardiac muscles therefore may be used to compare the degree of differentiation in normal and experimental muscles to get some insight into the factors affecting the process of differentiation. The thyroid hormones have been demonstrated to modulate changes in myosin isoforms [2-5] in these muscles. The present study was undertaken to determine whether hypothyroidism affects the transitions in the developmental isoforms of troponin T during development of either skeletal or cardiac muscle and if it leads to the re-expression of developmental isoforms in the adult striated muscles.

Materials and Methods

Hypothyroidism was induced *in utero* at 10 day gestation by adding propylthiouracil (PTU) to the drinking water (0.06mg/ml) of pregnant rats (white Wistar strain) and maintaining the nursing mothers and their pups on this regimen until 20, 30, 50 or 60 days after birth. In another set of rats, hypothyroidism was induced at the age of three months and maintained for a period of two months. After these time intervals, the control and experimental animals were weighed, killed and individual muscles collected. Muscle samples for the Western blots were prepared as described previously [8]. The Western blots of skeletal muscle samples were stained with antibody F24 that is specific for the fast class of troponin T [9]. The Western blots of cardiac muscles were stained with antibody CDC4 that recognises both the adult and embryonic cardiac troponin T [8]. The levels of thyroid hormones T3 and T4 in rat serum samples were measured according to the method of Black *et al.* [1].

Results

The levels of circulating thyroid hormones (T3 and T4) in sera of all rats and the pups treated with PTU were reduced (Tables 1 and 2). Hypothyroidism was also apparent from the reduced growth rates of these animals indicated by much lower body weights compared with the normals (Tables 1 and 2).

The study of four developing skeletal muscles namely Pectoral, Triceps, Biceps and Flexor digitorum sublimis (FDS), showed a reduction in the rate of foetal and neonatal

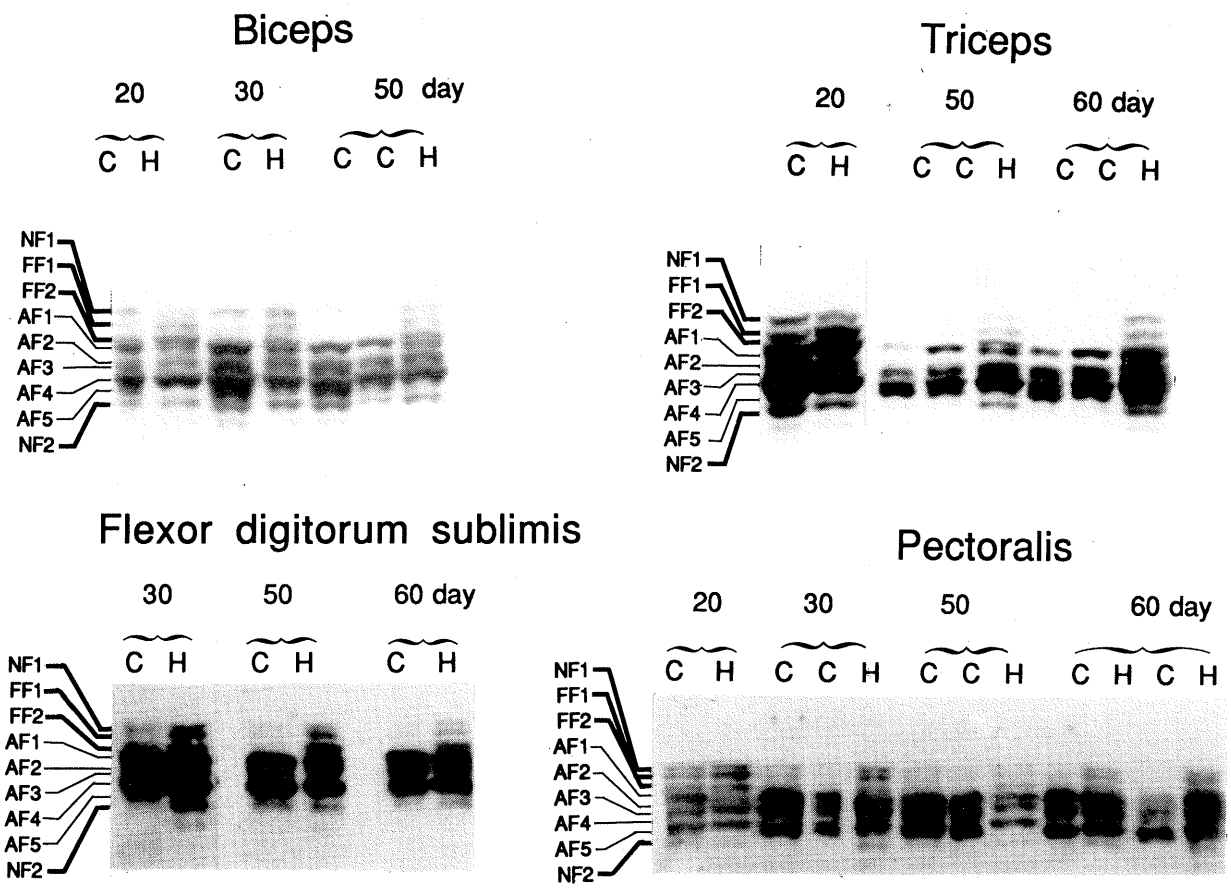

Figure 1. The expression of adult (AF1-AF5) and developmental isoforms (FF1, FF2, NF1 and NF2) of troponin T in some skeletal muscles of control (C) and hypothyroid (H) rats studied by the staining of Western blots with antibody F24. The hypothyroidism was induced at 10 days in utero and the muscles analysed 20-60 days after birth. For Triceps and Pectoral muscles, there are two control muscle samples for some stages. For Triceps, the sample on the left is deeper and on the right superficial part. For Pectoralis muscle, the sample on the left is P. major and on the right P. minor. For 60 day, samples for both control and hypothyroid on the left are P. major and on the right P. minor.

Table 1. Level of thyroid hormones and body weights of rats when PTU treatment was started at the age of 90 days and continued for 60 days.

	Control rats	PTU-treated rats
Weight (g)	459.4 ± 23 (n = 5)	395.2 ± 11.7 (n = 5)
T4 level (nmol/l)	51.20 ± 6.7 (n = 5)	< 10 (n = 5)
T3 level (nmol/l)	1.58 ± 0.23 (n = 5)	0.83 ± 0.30 (n = 5)

to adult isoform transitions (Fig. 1). For example, by day 30 (not shown), developmental isoforms in control Triceps muscle were present in only trace amounts and were undetectable by day 50 in both deeper and superficial regions of this muscle (Fig. 1). In contrast, in Triceps muscle from hypothyroid rats, all four developmental isoforms were still present by 60 days of age although the level of adult isoforms had gradually increased by this stage. In control Biceps, isoform FF1 was suppressed by day 20 and FF2 and NF1 by day 50 of age. In hypothyroid rats, NF1, FF1 and FF2 isoforms were still present in this muscle by 50 days of age. In control FDS muscle, isoforms NF1, FF1 and FF2 were present in only small amounts by day 30 and reduced to trace amounts by days 50-60 of age. In hypo-

Table 2. The level of thyroid hormones and body weights of developing rats when PTU treatment was started at 10 days in utero.

Age	Control rats Weight (g)	T4level nmol/l	T3 level nmol/l	Weight (g)	PTU-treated T4 level nmol/l	T3 level nmol/l
20	31.3 ± 0.58 (n = 3)	48 ± 4 (n = 3)	1.67 ± 0.19 (n = 3)	19.3 ± 1.7 (n = 3)	< 10 (n = 3)	0.87 ± 0.07 (n = 3)
30	87 ± 2.6 (n = 3)	43 ± 5 (n = 3)	1.87 ± 0.14 (n = 3)	21 ± 6 (n = 3)	< 10 (n = 3)	1.03 ± 0.31 (n = 3)
50	200 ± 20 (n = 3)	46 ± 4 (n = 3)	1.83 ± 0.25 (n = 3)	29.6 ± 1.6 (n = 3)	< 10 (n = 3)	0.73 ± 0.14 (n = 3)
60	268 ± 20.4 (n = 3)	48 ± 4 (n = 3)	1.70 ± 0.30 (n = 3)	48 ± 5 (n = 3)	< 10 (n = 3)	1.10 ± 0.42 (n = 3)

thyroid rats, the developmental isoforms in FDS muscle were still present in significant amounts by 60 days of age but the level of adult isoforms had increased. In the control Pect. minor, all four developmental isoforms were suppressed by day 30. In the control Pect. major, trace amounts persisted until day 50 but were barely detectable by day 60. In Pectoral muscles from hypothyroid rats, the developmental isoforms were still present by 60 days of age although the relative level of adult isoforms gradually increased.

In a second set of experiments, the effect of hypothyroidism was investigated in rats in which the transitions in the developmental isoforms have been completed to see if it resulted in the re-expression of developmental isoforms. Their pattern was also investigated in muscles in which trace amounts of some developmental isoforms normally persist in the adult to see if the level of these isoforms increased by hypothyroidism. This study showed that the induction of hypothyroidism did not result in either the re-expression of developmental isoforms in Biceps, Triceps and external or internal oblique muscles or any increases in their levels in Masseter, Temporalis or tongue muscles in which trace amounts of some developmental isoforms persist in the adult (not shown).

Hypothyroidism induced at the age of three months maintained for a period of two months also did not show the reexpression of embryonic cardiac troponin T in the rat hearts (not shown). The study of developing cardiac muscle when hypothyroidism was induced at 10 days *in utero* and the hearts analysed 20 days postnatally also did not show any changes in the rate of embryonic to adult cardiac troponin T transition in any of the four cardiac chambers examined (not shown).

Discussion

The reduction in circulating thyroid hormones reduced the rate of transitions of troponin T isoforms in developing skeletal muscles but it did not completely inhibit the expression of the adult fast isoforms the level of which although somewhat retarded nevertheless proceeded gradually. The reduction observed in the rate of suppression of developmental isoforms of troponin T in the present study may reflect the much lowered growth rates of these animals. Unlike troponin T, hypothyroidism during development has been reported to completely inhibit transition to adult myosin when the hypothyroid state is induced at 18 days *in utero* and examined 4 weeks after birth [2]. It is possible that the appearance of adult myosin in hypothyroid rats requires longer than 4 weeks [4]. The accumulation of all adult fast isoforms of troponin T observed in all skeletal muscles in the present study clearly differs from myosin. The study of adult skeletal muscles showing no apparent changes in troponin T also differed from myosin for which hypothyroidism has been reported to induce embryonic and neonatal myosin heavy chains in some adult rat skeletal muscles [5]. This difference in the isoforms of myosin and troponin T may be attributed to the possibility that while changes in the neonatal to adult myosins occur at gene level, transitions in the fast class of troponin T described here (comprising foetal, neonatal and adult fast) may arise due to alternative RNA splicing of the same gene. The myosin heavy chain genes have also been shown to contain thyroid responsive elements while this is not known for the troponin T genes.

The most striking difference of hypothyroidism in its effect on troponin T isoforms compared with myosin was observed for the cardiac muscle. Unlike the studies of myosin [3], reduction in the level of thyroid hormones did not show any changes in either the adult or developing rat hearts. These results are in agreement with those reported by Saggin *et al.* (10) who also did not observe any changes in the expression of foetal cardiac troponin T in developing hearts although the same samples showed changes in the ratios of α and β myosin heavy chains. As may also be the case for the fast skeletal troponin T, the changes in the cardiac troponin T are brought about by RNA splicing mechanism unlike cardiac myosin, V1 and V3 forms of which are encoded by distinct genes. This makes it tempting to speculate a role for thyroid hormones in regulating

some cell type and or developmental stage-specific proteins that control RNA splicing (6, 7).

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