Influence of thyroid hormone on the tissue-specific expression of cytochrome *c* oxidase isoforms during cardiac development

Julia MEEHAN and John M. KENNEDY

Department of Physiology and Biophysics, University of Illinois at Chicago, 835 S. Wolcott Ave (m/c 901), Chicago, IL 60612-3796, U.S.A.

In mammals, cytochrome *c* oxidase (COX) is composed of 13 different protein subunits. In the rat, two nuclear-encoded subunits, COX VIa and VIII, exist as tissue-specific isoforms: heart and liver. Using Northern-blot analysis, the levels of transcripts for the heart and liver isoforms of VIa and VIII were examined in developing rat hearts. The liver isoform was found to be the predominant form of subunit VIa and the exclusive form of VIII in the 18-day fetal hearts. The mRNA levels of the heart isoform of both subunits increased dramatically to reach adult levels by 14 days. Although the levels of the VIa- and VIII-liver isoform mRNAs remained stable throughout early development, their levels decreased by 40 and 36 % respectively between the 18-day fetal stage and 18-day neonatal stage. Therefore the up-regulation of the heart isoforms and down-regulation of the liver isoforms appear to be regulated in a co-ordinated manner during development. To determine if thyroid hormone influences the expression of these developmentally regulated isoforms, the RNA was also extracted from the hearts of 2-week-old hypothyroid rats. The results showed that the levels of VIII-heart and VIa-liver COX mRNAs were approx. 40 % lower in the hypothyroid hearts, while VIII-liver and VIa-heart COX isoform expression remained unchanged. These data demonstrate that the isoforms of COX subunits VIa and VIII are not co-ordinately regulated by changes in thyroid hormone levels. Therefore we conclude that, although thyroid hormone influences the expression of isoforms, it appears to do so via a different mechanism from that which regulates the developmental transition.

INTRODUCTION

During the first 11 days of life, a 5-fold increase in heart weight is observed in rats [1], illustrating the considerable growth that the mammalian heart undergoes during early neonatal development. Protein and mRNA concentrations [2,3] and the relative volume of myocytes, mitochondria and capillaries [1,4] increase greatly, resulting in the rapid growth of ventricular wall thickness and total myocardial mass. Between 10 and 16 days of postnatal life, the contractile aspects of cardiac muscle, including peak active tension, peak passive tension and rate of tension development, increase to such a degree that by 16 days old, the rat heart has reached functional and structural maturity, with no significant differences compared with the adult heart [5]. The mitochondria in the myocardium also increase in size and number, resulting in a 5 %/day increase in mitochondrial density over the first 3–5 weeks of development [1,6], and mitochondrial cristae appear denser and more organized [1,4,7]. In mitochondria, the terminal enzyme of the electron-transport chain, cytochrome *c* oxidase (COX) shows large increases in mRNA levels [8–10], protein content and enzyme activity [10–13] during development.

Mammalian COX is composed of 13 different polypeptide subunits; the three largest subunits are encoded by the mitochondrial genome and are responsible for the catalytic function of the enzyme. The function of the remaining ten nuclear-encoded subunits remains somewhat unclear, although recent reports suggest that they may play a role in regulating catalysis [14–16] or in the assembly of the enzyme complex [17,18]. In addition, some of the nuclear-encoded subunits have been found to exist as tissue-specific isoforms, heart and liver [19–21], named after the tissues where they were first identified. The rat genome encodes heart and liver isoforms for subunits VIa and VIII [20–24], and the human has isoforms for at least two subunits, VIa and VIIa [11]. Typically, the liver isoform is expressed in variable amounts in all tissues and the heart form is expressed only in the heart and skeletal-muscle tissues [11,24–27]. However, bovine intestinal smooth muscle [25] and rat brown fat [22] have been shown to also contain the heart form of subunits VIIa and VIII respectively. In the rat, the heart and liver isoforms of subunit VIII are only 39 % homologous. In contrast, comparisons of the rat VIII-heart and bovine VIII-heart isoform sequences show 56 % homology [23]. Therefore it has been suggested that tissue-specificity appears to over-ride species-specificity, implicating a tissue-specific function for the presence of different isoforms.

Thyroid hormone is known to stimulate mitochondrial biogenesis in most tissues [28–30], and an induced thyroid deficiency produces reductions in COX activities and mRNA levels in liver [29,31], cardiac [10,32,33] and skeletal muscle [10,29,34]. Furthermore thyroid hormone has previously been shown to regulate the expression of contractile proteins in muscle, causing a developmental switch of myosin heavy chain (MHC) isoforms in cardiac and skeletal muscle [35,36]. The switch of isoforms in rats is associated with the accumulation of serum thyroxine levels, starting at 2 days after birth [37] and reaching maximal levels by day 16. By 28 days after birth, the tri-iodothyronine form of thyroid hormone and the adult form of the heavy chain, α-MHC, reach mature levels in the rat [36,37]. It is not known whether thyroid hormone regulates or influences the transition of COX isoforms during development.

Developmental regulation of COX subunit isoforms has been observed in human [38], bovine [26] and mouse [9] hearts. In the heart tissue, there is a switch in gene expression from the liver form to the heart form as the animal progresses from the fetal to the adult stage. The mechanisms that may regulate this transition

Abbreviations used: COX, cytochrome *c* oxidase; MHC, myosin heavy chain.

have not yet been investigated. The experiments reported here were designed to determine the developmental time course of VIa and VIII isoform expression in the rat heart more precisely and to test the hypothesis that thyroid hormone initiates or influences the developmental switch of isoforms. The relative levels of heart and liver isoform mRNA were determined in late fetal to early neonatal heart development by Northern blotting in order to investigate developmentally regulated transitions in isoform expression. In addition, the amount of isoform transcripts in heart samples from 2-week-old hypothyroid and control rats were compared to evaluate the influence of thyroid hormone during this developmental period. Some results have previously been presented in preliminary form [39].

MATERIALS AND METHODS

Animal procedures

Under ether anaesthesia, hearts and other tissues were removed from 18- and 20-day fetal, 0.5-, 1.5-, 2.5-, 3-, 11-, 14-, 18-day-old neonatal, and adult Sprague–Dawley rats. Tissue samples to be used for RNA isolation were immediately frozen on dry ice and stored at −80 °C. In order to produce hypothyroid rats, timed-pregnant female Sprague–Dawley rats were obtained from Sasco (Omaha, NE, U.S.A.) and fed methimazole-treated water (0.025 %) starting on the 16th day of gestation, continuing through the weaning period. The length of rat gestation is 22 days. Methimazole is a drug that inhibits thyroid-gland function and passes through breast milk, inducing hypothyroidism in rat pups [40]. At 14 days old, the resulting hypothyroid neonates were killed by decapitation under ether anaesthesia. Tissues were collected and samples to be used for RNA extraction were frozen and stored at −80 °C. All animal procedures are in accordance with AALAC animal care policy.

Assay of COX activity

Fresh tissue samples were homogenized in 20 vol. of 0.1 M phosphate buffer, pH 7.0, to determine COX activity as described by Cooperstein and Lazarow [41]. This method utilizes a spectrophotometer and a kinetics analysis software program to measure the rate at which reduced cytochrome *c* is oxidized by the enzyme within the homogenate. The enzyme activity is expressed as the rate of oxidation per total protein present in the homogenate as determined by Lowry et al. [42].

Northern-blot analysis

Total RNA was isolated from frozen tissue by the method of Chomczynski and Sacchi [43]. Total RNA (15 μg) was separated in a 1.5 % agarose gel and transferred to a Zetaprobe nitrocellulose membrane (Bio-Rad, Richmond, CA, U.S.A.). Prehybridization of blots was carried out for 2–4 h at 42 °C in solution containing 40 % formamide, 5 × SSC (where 1 × SSC is 0.15 M NaCl/0.015 M sodium citrate), 10 × Denhardt's solution (where 1 × Denhardt's is 0.02 % Ficoll 400/0.02 % polyvinylpyrrolidone/0.02 % BSA), 50 mM sodium phosphate, pH 6.5, 0.2 % SDS and 0.5 mg/ml denatured salmon sperm DNA. Probes labelled with α-^{32}P-labelled dATP (Amersham, Arlington Heights, IL, U.S.A.) were prepared according to the protocol of a Random Priming kit (Gibco–BRL Life Technologies, Gaithersburg, MD, U.S.A.). The rat COVIII heart (280 bp) and liver (391 bp) cDNA probes were contributed by Dr. Bernard Kadenbach (Fachbereich Chemie der Philipps-Universität, Marburg, Germany). The human COVIa heart (371 bp) and liver (546 bp) cDNA probes were contributed by Dr. Margaret Lomax (University of Michigan, Ann Arbor, MI, U.S.A.). The α-MHC and β-MHC 3′ untranslated region cDNA probes were contributed by Dr. L. Leinwand (University of Colorado, Boulder, CO, U.S.A.). Hybridizations were carried out overnight at 42 °C in solution containing 40 % formamide, 3 × SSC, 2 × Denhardt's, 0.2 % SDS and 0.17 mg/ml denatured salmon sperm DNA. Blots were washed at room temperature in 2 × SSC/0.1 % SDS (4 × 15 min) and at 56 °C in 0.5 × SSC/0.1 % SDS (4 × 20 min). Autoradiography was performed at −80 °C, using Kodak XRP-5 film. Blots were stripped in 0.1 × SSC/0.5 % SDS at 100 °C for 30 min, and rehybridized. Densitometric scans of the autoradiographs were taken and the area of each band's peak was determined. The levels of transcripts were normalized relative to expression of the 18S rRNA. In the hypothyroid study (see Figure 5), two Northern blots, each with four samples from both hypothyroid and control rat hearts, totalling 16 samples altogether, were sequentially hybridized with isoform cDNAs. Each sample was densitometrically scanned, normalized to 18S rRNA, and the control values of one blot were normalized to the controls of the other; the combined hypothyroid results are given as a percentage of the control.

Statistics

Values are expressed as means ± S.E.M. Differences between groups were evaluated using Student's *t* test and accepted as significant if $P < 0.05$.

RESULTS

In this study, our goal was to show a developmental switch of tissue-specific isoforms of COX subunits VIa and VIII at the level of mRNA expression in the rat heart. We were able to confirm the tissue-specificity of the isoforms by performing Northern-blot analysis of various rat tissues. Similarly to previous findings in bovine [25,26,44], rat [22] and human [38], we found that the liver isoform of both subunits, VIII and VIa, was expressed at variable levels in liver, brain, lung, skeletal muscle, atria and ventricle tissues, whereas the heart isoform was expressed in the skeletal muscle, atria and ventricle tissues only (results not shown).

With tissue-specificity of the isoforms confirmed, their developmental expression was investigated. Total RNA samples extracted from fetal, neonatal and adult rat hearts were used for Northern-blot analysis. Virtually no VIII-heart isoform transcripts were detected in the 18-day fetal sample, but the level progressively increased to reach mature levels by 11 days (Figure 1A). The expression of the VIII-heart isoform showed a 10-fold increase in the relative transcript levels in the short period of development between fetal day 20 and neonatal day 14. A developmental increase of expression was also observed for the VIa-heart isoform. However, in contrast with the VIII-heart isoform, low levels of VIa-heart isoform transcripts were detected in the 18-day fetal heart (Figure 1B). Earlier time points were not examined, therefore it is not known whether there is a developmental age when the liver isoform of subunit VIa is the exclusive isoform expressed, as was the case for subunit VIII. The liver isoform of both subunits, VIa and VIII, was the predominant isoform expressed in the fetal hearts, and the relative level of mRNAs for the liver isoforms remained stable until neonatal day 14, but appeared to decrease in the 18-day-old neonatal and adult hearts (Figures 1A and 1B). Across this same time course, ventricular tissue showed a 5-fold increase in COX activity (Table 1).

To verify that no (VIII) or low (VIa) amounts of heart isoform

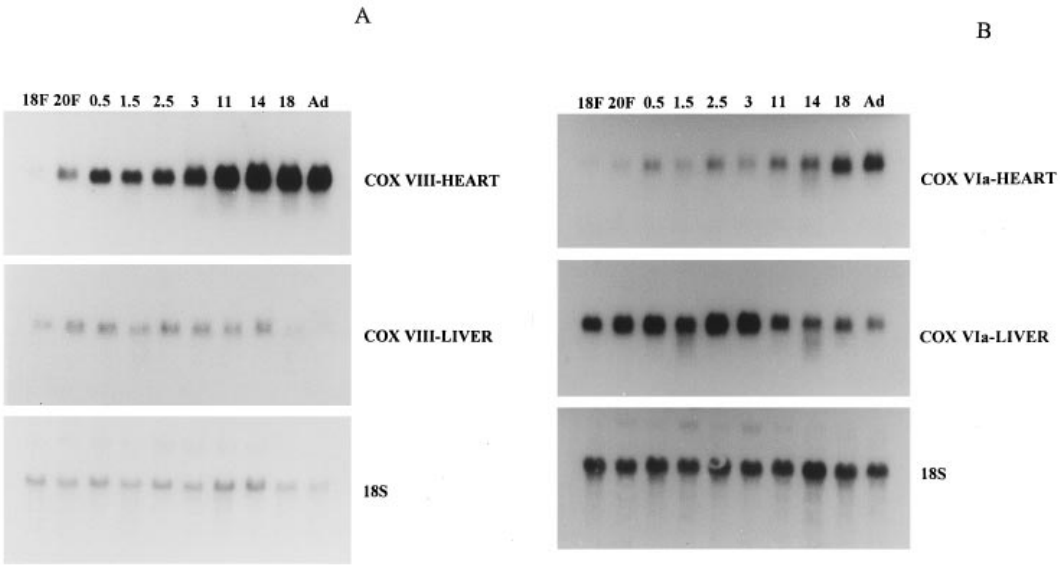

Figure 1 Developmental time course of COX isoform expression

Northern blots were sequentially hybridized with cDNA probes to detect VIII-heart and VIII-liver isoform mRNA and 18S rRNA (**A**) or VIa-heart and VIa-liver isoform mRNA and 18S rRNA (**B**). Ventricle tissues were removed from 18- and 20-day fetal hearts and 0.5-, 1.5-, 2.5-, 3-, 11-, 14-, 18-day-old neonatal hearts, and the adult sample (Ad) was obtained from post-partum female rats.

Table 1 Developmental time course of COX activity

Enzyme activity of ventricular tissue homogenates was measured by spectrophotometry. Adult hearts were obtained from post-partum female rats. Results are means ± S.E.M. for the number of samples given in parentheses.

Age	COX activity (nmol/min per μg of protein)
Fetal day 18	223.14 ± 31.10 (3)
Fetal day 20	340.95 ± 47.51 (3)
0.5 days	378.31 ± 40.83 (2)
1.5 days	437.73 ± 11.78 (2)
2.5 days	487.99 ± 15.52 (2)
3 days	743.40 ± 162.92 (3)
11 days	654.47 ± 44.29 (2)
14 days	862.90 ± 300.80 (2)
18 days	682.74 ± 78.11 (3)
Adult	1052.71 ± 86.00 (4)

transcripts are expressed in 18-day fetal hearts and that the liver isoforms are indeed down-regulated in 18-day-old neonatal and adult hearts, statistical analysis of the isoform transition was performed using total RNA from four 18-day fetal and four 18-day-old neonatal heart samples. No VIII-heart isoform was detected in any of the four fetal heart samples, whereas relatively large amounts were detected in the neonatal samples (Figure 2A). Transcripts of the VIII-liver isoform were detected in hearts from both age groups, but the relative level of the liver transcript in the 18-day-old neonatal hearts was 36% less ($P < 0.05$) than in the 18-day fetal hearts, where the liver form was the only form of subunit VIII expressed.

A similar pattern of expression was observed for the VIa isoforms in Figure 2(B). However, unlike the VIII-heart isoform, VIa-heart isoform transcripts were detected at low levels in all 18-day fetal heart samples, and increased 5.5-fold ($P < 0.01$) between the 18-day fetal stage and the 18-day-old neonatal stage. The VIa-liver isoform was the predominant form of subunit VIa in fetal hearts, but in neonatal hearts its expression was down-regulated by 40% ($P < 0.05$). These results quantitatively substantiate the developmental up-regulation of heart isoforms and the down-regulation of liver isoforms that was depicted in Figure 1. Although direct comparisons cannot be made between the levels of VIa and VIII tissue-specific isoform expression, the pattern of the isoform transition is very similar, suggesting that the mechanisms that regulate expression of these genes converge so that they are co-ordinately regulated.

The regulatory mechanism involved in the developmental transition of COX isoforms in the rat heart is unknown. One possibility may involve thyroid hormone which is known to activate mitochondrial biogenesis [28–30,45]. Thyroid hormone also causes the developmental transition of rat cardiac MHC isoforms by up-regulating transcription of the mRNA of α-MHC and down-regulating transcription of the β-MHC isoform [36]. In order to demonstrate whether thyroid hormone is involved in the regulation of COX isoforms in the rat heart, we used a hypothyroid rat model. It has been determined in the rat that circulating thyroid hormone levels begin to rise at birth, surge 4 days after birth, and reach mature levels by 16 days postnatally [37]. If thyroid hormone does indeed regulate the developmental transition of the VIa and VIII isoforms, then the hearts from hypothyroid neonates should express the liver form predominantly, as in the 18-day fetal hearts. The reduced body weights of the 14-day-old methimazole-treated rats (31.25 ± 1.93 g) compared with the control rats (47.5 ± 0.74 g) and the re-expression of the β-MHC mRNA in hypothyroid heart samples (Figure 3) clearly demonstrate that hypothyroidism was induced. Figure 4 shows a representative Northern blot comparing the expression of VIa and VIII isoforms

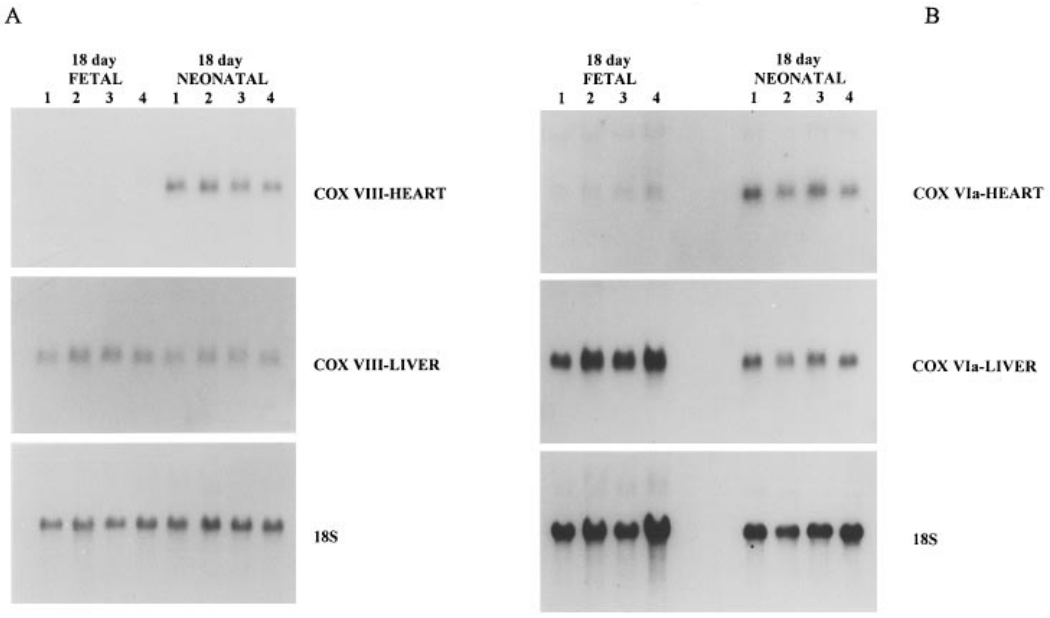

Figure 2 Comparison of fetal and neonatal isoform expression

Northern-blot analysis compares two age groups ($n = 4$). Each of the four samples from 18-day fetal hearts represents 8–13 hearts pooled from a single litter. Each ventricular neonatal sample is from a single heart of an 18-day-old rat. Blots were sequentially hybridized with either VIII-heart, VIII-liver and 18S rRNA probes (**A**) or VIa-heart, VIa-liver and 18S rRNA probes (**B**). The relative levels of VIII-liver and VIa-liver isoforms in neonatal hearts were determined by densitometry and were 36 and 40% less respectively than in fetal hearts ($P < 0.05$).

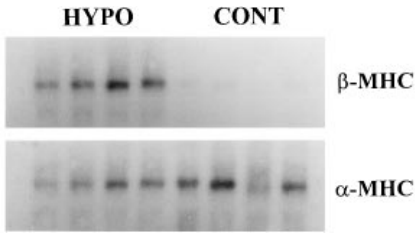

Figure 3 MHC transition in hypothyroid rats

A Northern blot comparing 14-day-old control (CONT) and hypothyroid (HYPO) rat hearts ($n = 4$) was analysed with α-MHC and β-MHC probes. The level of β-MHC transcripts in hypothyroid rats was 15-fold higher than in controls.

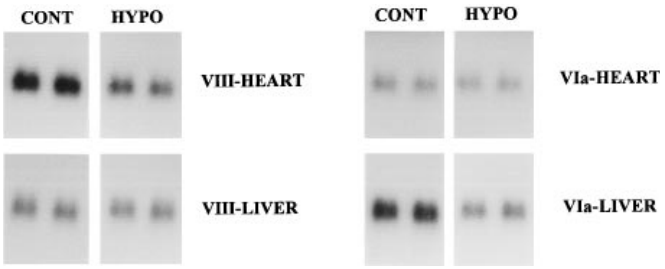

Figure 4 Isoform mRNA expression in hypothyroid and control rat heart

Representative Northern blot comparing 14-day-old hypothyroid and control rat hearts was sequentially hybridized with VIII-heart, VIII-liver, VIa-heart and VIa-liver cDNA probes. The first two lanes of the blot (CONT) contain heart RNA from two control rats; HYPO is heart RNA from two hypothyroid rats.

in hypothyroid and control samples. In hearts from 14-day-old hypothyroid rats, the VIII-liver isoform expression was not different from controls, whereas the VIII-heart isoform was expressed at only 61% of control levels ($P < 0.001$). For subunit VIa, on the other hand, the expression of the heart isoform in hypothyroid hearts was no different from controls, but the liver isoform was reduced in hypothyroid hearts to 58% of controls ($P < 0.001$). Therefore the removal of thyroid hormone produced different expression patterns for the two different isoforms, indicating that the regulation of the tissue-specific isoforms of these two subunits can be uncoupled.

DISCUSSION

In this study, we demonstrate that the mRNA expression of tissue-specific isoforms of COX VIa and VIII is developmentally regulated in the rat heart. The heart isoforms of both VIa and VIII increase dramatically as development progresses from the fetal to the adult stage. Although the expression of the heart isoform has previously been shown to be highly up-regulated in bovine (VIa) and human (VIa and VIIa) hearts during development, the fetal hearts in these species already expressed the heart isoform in relatively large amounts [26,38], whereas our results in the rat demonstrated that the fetal heart expressed little (VIa) or no (VIII) heart isoform. The liver isoform was the predominant form of VIa and VIII in the fetal rat heart followed by a significant down-regulation as the rat approached adulthood. In contrast, in the bovine [26] and the human fetal heart [38], the VIa-liver isoform constituted only 25 and 30% respectively, of the total amount of subunit VIa message. In summary, we determined quantitatively that there is an isoform transition in the rat heart during development, and the degree of

this transition is much greater than that observed in other species.

The function(s) of tissue-specific isoforms is currently under investigation. There is some preliminary evidence that the COX enzyme from bovine liver has a higher V_{max} and a lower K_m than the enzyme isolated from the bovine heart [21], although this result has not been reproducible by others [46]. In addition, subunit VIa isolated from bovine heart was shown to bind the nucleotide, ADP [14], which exhibited a stimulatory effect on the activity of the COX enzyme from heart tissue [15], whereas the enzyme from the bovine liver showed no such ADP binding or stimulation. These observations suggest that the VIa-heart isoform may provide a mechanism by which the COX enzyme in striated muscle can respond to changes in the energy state of the tissue, thus inferring a functional significance for tissue-specific isoforms. The COX enzyme of a strictly aerobic slime mould, *Dictyostelium discoideum*, switches isoforms of subunit VII, from VIIe to VIIs, when exposed to a hypoxic environment [47]. The level of VIIs expression parallels the degree of hypoxia, suggesting a physiological significance for the switching of isoforms in the respiratory enzyme of this species.

The regulation of tissue-specific COX isoform expression in mammals has yet to be elucidated. Thyroid hormone is known to enhance mitochondrial biogenesis [28–30] by stimulating transcription of different nuclear-encoded mitochondrial proteins, such as cytochrome aa_3, cardiolipin [48], cytochrome *c*, adenine nucleotide translocator 2 and F_1-ATPase subunit *c* [49]. Also, in hypothyroid rats, the relative heart weights and the myofibrillar ATPase, citrate synthase [6] and COX [10,33] enzyme activities in heart are significantly reduced. We utilized a method for inducing hypothyroidism in neonatal rats to investigate the role, if any, that thyroid hormones play in the developmental switching of tissue-specific isoforms in the heart. We are the first to examine the effects of thyroid hormone on the regulation of tissue-specific COX isoforms. Our results show that, in the absence of thyroid hormone, the expression of VIII-heart and VIa-liver isoforms is reduced, whereas the expression of VIII-liver and VIa-heart isoforms is not affected. Rat VIII-heart [50], VIa-heart and VIa-liver isoform genes [51] have been recently sequenced and do not appear to contain a thyroid hormone-response element in their promoter regions. Therefore it is unlikely that thyroid hormone regulates the transcription of these genes directly; it appears to affect a more distal transcription factor. Thyroid hormone appears to disrupt the apparent developmental co-regulation of the tissue-specific isoforms so that there is relatively more of the heart isoform of subunit VIa and more liver isoform of subunit VIII in hypothyroid animals. These data indicate that the expression of these COX subunits is subject to different regulatory pathways for each set of tissue-specific isoforms. Different regulatory pathways may also be inferred from the existence of different COX isoenzymes in rat brown fat and bovine smooth muscle. COX enzyme composition in rat brown fat includes VIa-liver and VIII-heart isoforms [22]. COX isolated from bovine smooth muscle includes the VIa-liver, VIIa-heart and both VIII-liver and VIII-heart isoforms [25]. The fact that these COX isoenzymes contain various isoform combinations indicates that different regulatory pathways must exist for each tissue-specific subunit. The results of the present study demonstrate that thyroid hormone may influence the patterns of isoform expression via these divergent pathways. Altering the thyroid status has previously been shown to disrupt the coordinated regulation of the mitochondrial- and nuclear-encoded COX subunits [28,29,34]. Hypothyroidism induces large decreases in the mRNA levels of only three COX subunits, II, III and Va, in skeletal muscle. In contrast, in the liver, hypothyroidism produces small decreases in mRNA levels of mitochondrial-encoded subunits II and III, while causing large decreases in the level of the nuclear-encoded VIc mRNA [29], indicating that this disparate regulation of COX subunits by thyroid hormone appears to be tissue-specific as well.

In the present study, development of the rat heart from the late fetal to early neonatal stage produced large increases in COX activity and COX heart isoform transcript levels. A developmental transition of VIa and VIII isoforms, from liver to heart, in the rat heart was also demonstrated. The demand for a higher level of cardiac contractility in the neonatal and adult heart may provide the impetus for the switch to the heart isoforms, which may display a higher level of energy efficiency as discussed by Rohdich and Kadenbach [15]. Thyroid hormone is known to regulate the expression of other respiratory-chain transcripts and induce a switch between the α and β subunits of the heart contractile protein MHC. We have demonstrated that it also influences the expression of the VIII-heart and VIa-liver isoforms, but does not appear to direct the switch of the heart and liver transcripts that is observed across development, since the expression of the VIII-heart subunit is not completely turned off and the expression of the VIa-heart subunit is completely unaffected by the absence of thyroid hormone.

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REFERENCES

1 Anversa, P., Olivetti, G. and Loud, A. V. (1980) Circ. Res. **46**, 495–512
2 Peterson, C. J., Whitman, V., Watson, P. A., Schuler, H. G. and Morgan, H. E. (1989) Circ. Res. **64**, 360–369
3 Whitsett, J. A. and Darovec-Beckerman, C. (1981) Pediatr. Res. **15**, 1363–1369
4 Smith, H. E. and Page, E. (1977) Dev. Biol. **57**, 109–117
5 Hopkins, S. F., McCutcheon, E. P. and Wekstein, D. R. (1973) Circ. Res. **32**, 685–691
6 Dowell, R. T., Haithcoat, J. L. and Hasser, E. M. (1983) Proc. Soc. Exp. Biol. Med. **174**, 368–376
7 Sordahl, L. A., Crow, C. A., Kraft, G. H. and Schwartz, A. (1972) J. Mol. Cell. Cardiol. **4**, 1–10
8 Hevner, R. F. and Wong-Riley, M. T. T. (1993) J. Neurosci. **13**, 1805–1819
9 Kim, K., Lecordier, A. and Bowman, L. H. (1995) Biochem. J. **306**, 353–358
10 Stevens, R. J., Nishio, M. L. and Hood, D. A. (1995) Mol. Cell. Biochem. **143**, 119–127
11 Kennaway, N. G., Carrero-Valenzuela, R. D., Ewart, G., Balan, V. K., Lighthowlers, R., Zhang, Y.-Z., Powell, B. R., Capaldi, R. A. and Buist, N. R. M. (1990) Pediatr. Res. **28**, 529–535
12 Kennedy, J. M., Kelley, S. W. and Meehan, J. (1993) J. Mol. Cell. Cardiol. **25**, 117–131
13 Marin-Garcia, J. and Baskin, L. S. (1989) Pediatr. Cardiol. **10**, 212–215
14 Anthony, G., Reimann, A. and Kadenbach, B. (1993) Proc. Natl. Acad. Sci. U.S.A. **90**, 1652–1656
15 Rohdich, F. and Kadenbach, B. (1993) Biochemistry **32**, 8499–8503
16 Taanman, J.-W., Turina, P. and Capaldi, R. A. (1994) Biochemistry **33**, 11833–11841
17 Aggeler, R. and Capaldi, R. A. (1990) J. Biol. Chem. **265**, 16389–16393
18 Poyton, R. O., Trueblood, C. E., Wright, R. M. and Farrell, L. E. (1988) Ann. N. Y. Acad. Sci. **550**, 289–307
19 Hood, D. A., Zak, R. and Pette, D. (1989) Eur. J. Biochem. **179**, 275–280
20 Kuhn-Nentwig, L. and Kadenbach, B. (1985) Eur. J. Biochem. **149**, 147–158
21 Merle, P. and Kadenbach, B. (1982) Eur. J. Biochem. **125**, 239–244
22 Kadenbach, B., Stroh, A., Becker, A., Eckerskorn, C. and Lottspeich, F. (1990) Biochim. Biophys. Acta **1015**, 368–372
23 Scheja, K. and Kadenbach, B. (1992) Biochim. Biophys. Acta **1132**, 91–93
24 Schlerf, A., Droste, M., Winter, M. and Kadenbach, B. (1988) EMBO J. **7**, 2387–2391
25 Anthony, G., Stroh, A., Lottspeich, F. and Kadenbach, B. (1990) FEBS Lett. **277**, 97–100
26 Ewart, G. D., Zhang, Y.-Z. and Capaldi, R. A. (1991) FEBS Lett. **292**, 79–84
27 Van Kuilenburg, A. B. P., Muijsers, A. O., Demol, H., Dekker, H. L. and Van Beeumen, J. J. (1988) FEBS Lett. **240**, 127–132
28 Luciakova, K. and Nelson, B. D. (1992) Eur. J. Biochem. **207**, 247–251

29 Weisner, R. J., Kurowski, T. T. and Zak, R. (1992) Mol. Endocrinol. **6**, 1458–1467
30 Winder, W. W. (1979) Am. J. Physiol. **236**, C132–C138
31 Mutvei, A., Kuzela, S. and Nelson, B. D. (1989) Eur. J. Biochem. **180**, 235–240
32 Baldwin, K. M., Campbell, P. J., Hooker, A. M. and Lewis, R. E. (1979) Am. J. Physiol. **236**, C30–C34
33 Paradies, G., Ruggiero, F. M., Dinoi, P., Petrosillo, G. and Quagliariello, E. (1993) Arch. Biochem. Biophys. **307**, 91–95
34 Hood, D. A., Simoneau, J.-A., Kelly, A. M. and Pette, D. (1992) Am. J. Physiol. **263**, C788–C793
35 Chizzonite, R. A., Everett, A. W., Clark, W. A., Jakovcic, S., Rabinowitz, M. and Zak, R. (1982) J. Biol. Chem. **257**, 2056–2065
36 Lompre, A.-M., Nadal-Ginard, B. and Mahdavi, V. (1984) J. Biol. Chem. **259**, 6437–6446
37 Dussault, J. H. and Labrie, F. (1975) Endocrinology **97**, 1321–1324
38 Bonne, G., Seibel, P., Possekel, S., Marsac, C. and Kadenbach, B. (1993) Eur. J. Biochem. **217**, 1099–1107
39 Meehan, J. and Kennedy, J. M. (1994) FASEB J. **8**, (abstr.) A843
40 Cooper, D. S., Kieffer, J. D., Saxe, V., Mover, H., Maloof, R. and Ridgeay, E. C. (1984) Endocrinology **114**, 786–793
41 Cooperstein, S. J. and Lazarow, A. (1951) J. Biol. Chem. **189**, 665–670
42 Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
43 Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159
44 Lighthowlers, R., Ewart, G., Aggeler, R., Zhang, Y.-Z., Calavetta, L. and Capaldi, R. A. (1990) J. Biol. Chem. **265**, 2677–2681
45 De Leo, T., Di Maio, V., Di Meo, S. and Valente, M. (1985) J. Physiol. (London) **369**, 187P
46 Van Beeumen, J. J., Van Kuilenburg, A. B. P., Van Bun, S., Van den Bogert, C., Tager, J. M. and Muijsers, A. O. (1990) FEBS Lett. **263**, 213–216
47 Sandona, D., Gastaldello, S., Rizzuto, R. and Bisson, R. (1995) J. Biol. Chem. **270**, 5587–5593
48 Jakovcic, S., Swift, H. H., Gross, N. J. and Rabinowitz, M. (1978) J. Cell Biol. **77**, 887–901
49 Nelson, B. D., Luciakova, K., Li, R. and Betina, S. (1995) Biochim. Biophys. Acta **1271**, 85–91
50 Lenka, N., Basu, A., Mullick, J. and Avadhani, N. G. (1996) J. Biol. Chem. **271**, 30281–30289
51 Mell, O. C., Siebel, P. and Kadenbach, B. (1994) Gene **140**, 179–186

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