J. Membrane Biol. 119, 171–177 (1991)
002226319100016Q

The Journal of
Membrane Biology
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Thyroid Hormone Regulation of Na,K-ATPase Subunit-mRNA Expression in Neonatal Rat Myocardium

Jivan Melikian and Faramarz Ismail-Beigi
Department of Medicine, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Summary. Regulation of Na,K-ATPase $mRNA_\alpha$ isoform and $mRNA_\beta$ expression by thyroid hormone (T_3) in neonatal rat myocardium was examined. In euthyroid neonates between ages of 2 and 5 days, $mRNA_{\alpha 1}$, $mRNA_{\alpha 3}$, and $mRNA_{\beta 1}$ abundances were nearly constant while $mRNA_{\alpha 2}$ was undetectable. During the interval between postnatal days 5 and 15, $mRNA_{\alpha 3}$ decreased to negligible levels and $mRNA_{\alpha 2}$ became expressed and increased in abundance to account for ~20% of the $mRNA_\alpha$ pool by the 15th postnatal day. To examine the effect of T_3 on this developmental program, neonates were injected with 75 μg T_3/100 g body weight or diluent alone on the second and third postnatal days and myocardial Na,K-ATPase subunit-mRNA abundances were determined on the third and fourth postnatal days. Because T_3 treatment increased the RNA/DNA ratios of myocardial tissue, the subunit-mRNA abundances were normalized per unit DNA. Following 24 and 48 hr of T_3 treatment, the abundances of $mRNA_{\alpha 1}$, $mRNA_{\alpha 3}$, and $mRNA_{\beta 1}$ increased, while $mRNA_{\alpha 2}$ continued to remain undetectable during the 2-day interval between the second to fourth postnatal days. It is concluded that T_3 augments the abundance of Na,K-ATPase subunit mRNAs that are already being expressed in the neonatal rat myocardium. The results further suggest that T_3 does not act as a "molecular switch" in the developmental expression of the $mRNA_\alpha$ isoforms in rat myocardium during the first four postnatal days.

Key Words T_3 · Na,K-ATPase mRNA isoforms · developmental regulation of Na,K-ATPase

Introduction

Na^+,K^+-activated adenosine triphosphatase (Na,K-ATPase; Na,K-pump) is an integral plasma membrane enzyme through whose function intracellular Na^+ and K^+ concentrations are maintained [21]. The enzyme consists of two dissimilar subunits, α and β, that are present in equimolar ratio in purified preparations isolated from a variety of tissues [21]. The α, or "catalytic," subunit of Na,K-ATPase has an M_r of ~110,000 and is present as three isoforms designated α_1, α_2, and α_3 that are expressed in a tissue-specific manner [8, 29, 34, 35, 43, 46]. The β subunit of Na,K-ATPase has a M_r of ~55,000 and is heavily glycosylated [21, 36]. Recently a putative isoform of the β subunit mRNA (designated β_2) has been described, and the corresponding polypeptide is expressed in rat brain but not in rat myocardium [26, 38]. Isoforms of Na,K-ATPase exhibit different sensitivities towards cardiac glycosides such as ouabain; the α_1 isoform of the rat is least sensitive with a K_I of $\sim 10^{-4}$ M for ouabain, while both the rat α_2 and α_3 isoforms exhibit K_I's of $\sim 10^{-7}$ M for ouabain [1, 11, 14–16, 25, 34, 40, 41, 44]. Rat kidney and liver express the α_1 isoform almost exclusively, while both α_1 and α_2 isoforms are expressed in cardiac and skeletal muscle [8, 11, 16, 18, 29, 41, 46]. All three α-isoforms are expressed in rat cerebral cortex [8, 29, 34, 35, 43, 46]. In a recent study on the developmental expression of Na,K-ATPase subunit mRNAs in several rat tissues, Orlowski and Lingrel [29] reported that $mRNA_{\alpha 2}$ is not expressed in the myocardium at birth, while $mRNA_{\alpha 1}$ and lesser quantities of $mRNA_{\alpha 3}$ are present. By the 10th to 14th day of postnatal life, however, $mRNA_{\alpha 3}$ virtually disappears, while $mRNA_{\alpha 2}$ becomes detectable between the fifth to seventh postnatal days and reaches near-adult levels by day 28 at which time it remains a minor component of myocardial $mRNA_\alpha$ pool.

Triiodothyronine (T_3) stimulates Na,K-ATPase activity in a variety of mammalian target tissues including the myocardium [2, 4, 10, 11, 14, 17, 18, 22, 23, 27, 32]. The enhancement of enzyme activity is attributable to an increase in the number of enzyme units through increased biosynthesis of the enzyme [2, 22–24]. T_3 augments the abundance of the mRNAs encoding the subunits of the enzyme in several target tissues including myocardium, an effect that precedes the stimulation of Na,K-ATPase activity and in part results from enhanced transcription of the corresponding genes [2, 9–11, 18, 27]. We have recently reported that while the α_2-isoform and

$mRNA_{\alpha 2}$ are minor components of adult rat myocardial Na,K-ATPase and $mRNA_{\alpha}$ pools, respectively, the abundance of $mRNA_{\alpha 2}$ and high-ouabain-sensitivity enzyme activity are preferentially augmented by T_3 [11]. A near-selective T_3-induced increase in the high-ouabain-sensitivity form of the enzyme has additionally been reported in adult rat diaphragm [14].

In view of the transcriptional regulation of Na,K-ATPase by T_3, the preferential enhancement of $mRNA_{\alpha 2}$ and α_2-isoform by T_3 in rat myocardium and skeletal muscle [10, 11, 14, 18], and the developmental program of Na,K-ATPase $mRNA_{\alpha}$-isoform expression in the myocardium during the early postnatal period [29], we examined the possibility that the "molecular switching" between $mRNA_{\alpha 2}$ and $mRNA_{\alpha 3}$ is mediated by thyroid hormone. This possibility was further suggested by the observations that thyroid hormone concentrations in rat plasma at birth are negligible, and that T_4 and T_3 concentrations increase during the first few days postnatally to reach $\frac{1}{3}$ to $\frac{1}{2}$ adult concentrations by the seventh to tenth day [6, 7, 45], an interval during which the aforementioned changes in Na,K-ATPase $mRNA_{\alpha}$-isoform expression are manifested. The hypothesis was also consistent with the finding that T_3 can act as a "molecular switch" in the expression of the myosin heavy chain isoforms [12, 13, 19, 33]. In the present study we document the effects of T_3 on Na,K-ATPase $mRNA_{\alpha}$-isoform and $mRNA_{\beta 1}$ expression in euthyroid neonatal rat myocardium during the first four postnatal days. We find that T_3 augments the abundance of the subunit mRNAs that are already being expressed in this tissue, and that the hormone does not appear to act as a "molecular switch" in myocardial Na,K-ATPase $mRNA_{\alpha}$-isoform expression.

Materials and Methods

Materials

Standard compounds including 3,3′,5-triiodothyronine (T_3) were obtained from Sigma Chemical ^{32}P-α-TTP (3000 Ci/mmol) was purchased from Amersham. Nitrocellulose paper (BA-85) and "nick-translation" kits were obtained from Schleicher and Schuell and Bethesda Research Laboratories, respectively.

Methods

Animals and Preparation of Tissues

Litters were obtained on the first postnatal day and the mothers maintained on regular rat chow. In experiments designed to study the expression of Na,K-ATPase $mRNA_{\alpha}$ isoforms during development, noninjected neonates were studied up to 15 days of age. In experiments examining the effects of thyroid hormone, each litter was randomly assigned to diluent or T_3 groups and kept in separate cages. Beginning on the second postnatal day, neonates were injected subcutaneously with 75 μg T_3/100 g body weight [28] dissolved in 200 μl of 1 mM NaOH solution or diluent alone once daily and were sacrificed on postnatal days 3 and 4. Hearts were rapidly removed and frozen in liquid nitrogen and stored for less than two weeks prior to isolation of RNA.

Quantitation of Tissue Protein, RNA, and DNA

Three to five hearts were pooled at each time point and for each experimental condition. Methods previously described were employed without modification [2, 10].

Isolation of RNA and Analysis of Na,K-ATPase Subunit-mRNA Abundances

In each experiment 4–6 hearts were pooled for the isolation of RNA at each time point from the T_3- and diluent-treated neonates. Total RNA was isolated by ultracentrifugation as described by Chirgwin et al. [3]. Quantitation of RNA, electrophoresis, blotting, hybridization, and washing conditions were as described previously [2, 11, 42]. Approximately 20 μg of total RNA isolated from each of the different treatment groups was loaded per lane in four replicate blots were hybridized with plasmids containing full-length $cDNA_{\alpha 1}$, $cDNA_{\alpha 2}$, $cDNA_{\alpha 3}$, and $cDNA_{\beta 1}$ "nick-translated" to near-equivalent specific activity ($\sim 5 \times 10^8$ cpm/μg DNA; $\sim 3 \times 10^7$ cpm used per blot) [35, 36]. Following autoradiography, the intensity of the corresponding bands were determined by densitometry [2]. Ethidium-bromide staining of the ribosomal RNA bands was monitored on the gels and on the nitrocellulose papers to ensure equal RNA loading of the gels and complete transfer of the RNA [9, 11, 47].

To measure the relative abundances of Na,K-ATPase subunit mRNAs, autoradiograms were prepared at various intervals to ensure that the signals were in the linear response range of the film. The resulting density readings were corrected for the elapsed time of exposure, length of the cDNAs [35, 36], and the amount of radioactivity used in each hybridization reaction.

Statistical Analysis

Data from the different independent experiments were averaged and are presented as means ±SE. Analysis of variance and least-square difference between the means was used throughout to test for statistical significance [39]. *P* values less than 0.05 were taken as significant.

Results

A Northern blot demonstrating the expression of $mRNA_{\alpha 1}$, $mRNA_{\alpha 3}$, and $mRNA_{\beta 1}$ in 2-day-old and $mRNA_{\alpha 2}$ in 15-day-old euthyroid rat myocardial tis-

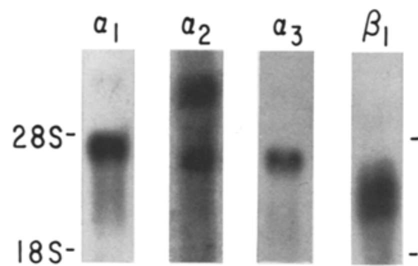

Fig. 1. Expression of Na,K-ATPase α_1-, α_3-, and β-mRNA in 2-day-old and of α_2-mRNA in 15-day-old euthyroid rat myocardium. RNA was isolated from 4–6 pooled neonatal rat hearts on the second or fifteenth postnatal days. Twenty μg of total RNA was fractionated, blotted, and probed with Na,K-ATPase cDNAs. Blots probed with $cDNA_{\alpha1}$, $cDNA_{\alpha3}$, and $cDNA_{\beta1}$ were from 2-day-old neonates. Note that the myocardial RNA probed with $cDNA_{\alpha2}$ was obtained from 15-day-old neonates. The positions of the 28 S and 18 S ribosomal-RNA bands are shown

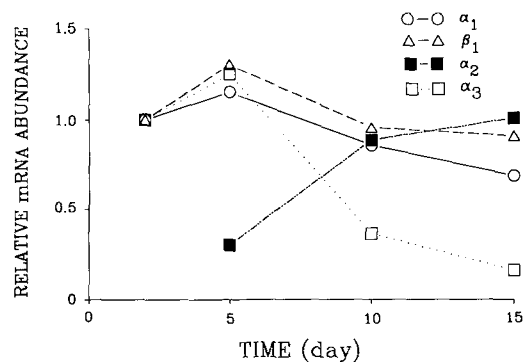

Fig. 2. Relative abundance of Na,K-ATPase subunit-mRNA isoforms in 2-day-old to 15-day-old euthyroid neonatal rat myocardium. RNA was isolated from 3–6 pooled neonatal rat hearts on the days indicated. Approximately 10 μg of total RNA from each time point was fractionated in adjacent lanes in four replicate blots and probed with full-length $cDNA_{\alpha1}$, $cDNA_{\alpha2}$, $cDNA_{\alpha3}$, and $cDNA_{\beta1}$. The experiment was repeated four times and the results averaged. To express the relative abundances of the mRNAs per unit DNA at each of the time points, the abundances were multiplied by the RNA/DNA ratio at the corresponding time points; RNA/DNA ratios were 0.69, 0.77, 1.04, and 0.83 on days 2, 5, 10, and 15, respectively. The resulting mRNA abundances were then normalized to their corresponding day-2 values except for $mRNA_{\alpha2}$ abundance on day 15 which was set to 1.0. $mRNA_{\alpha2}$ abundance on day 2 was low and could not be reliably determined. None of the changes in $mRNA_{\alpha1}$ and $mRNA_{\beta1}$ abundances were significant. $mRNA_{\alpha2}$ abundance on day 5 was significantly lower than its value on days 10 and 15, while $mRNA_{\alpha3}$ abundances on days 10 and 15 were significantly lower than the values on days 2 and 5

Table 1. Relative abundances of Na,K-ATPase $mRNA_{\alpha}$ isoforms and $mRNA_{\beta1}$ in myocardium of euthyroid neonatal rats on the second postnatal day

mRNA	Relative molar abundance
$mRNA_{\alpha1}$	1.0 ± 0.1
$mRNA_{\alpha2}$	"undetectable"
$mRNA_{\alpha3}$	0.4 ± 0.1
$mRNA_{\beta1}$	1.9 ± 0.1

Three samples of RNA were isolated from pooled myocardial tissue of 2-day-old euthyroid neonates from three separate litters. Twenty μg of total RNA obtained from each of the pooled myocardial samples were fractionated in 1% agarose gels in four replicate gels and following blotting were probed with $cDNA_{\alpha1}$, $cDNA_{\alpha2}$, $cDNA_{\alpha3}$, and $cDNA_{\beta1}$, respectively. The intensities of the resulting bands on the autoradiograms, corrected for the specific activity of the respective probes, the length of the cDNAs, and the period of exposure, were normalized to the mean intensity obtained utilizing the $cDNA_{\alpha1}$ probe. The SE of $mRNA_{\alpha1}$ value reflects the variance of the abundances in the three RNA samples. $mRNA_{\alpha2}$ abundance was below the limit of reliable detection. The results are expressed as means ± SE (n = 3).

sue is shown in Fig. 1. In the figure, RNA obtained from 15-day-old rats was used for $mRNA_{\alpha2}$ analysis because, as will be noted below, this mRNA species is not detectable in 2-day-old rat myocardium. The positions of the mRNA bands are in accordance to previous reports [11, 29, 35, 36, 46]. The relative abundances of α_1-, α_2-, α_3-, and β_1-mRNA in myocardium of 2-day-old euthyroid neonates were then determined. Replicate blots prepared from RNA isolated from three different litters were probed with the corresponding cDNAs and the relative density readings of autoradiograms determined (Table 1). On the second postnatal day, $mRNA_{\alpha1}$ abundance exceeded $mRNA_{\alpha3}$ abundance by 2.5-fold and $mRNA_{\alpha2}$ was undetectable; $mRNA_{\beta1}$ abundance exceeded the total $mRNA_{\alpha}$ pool by ~1.4-fold. On prolonged exposure (5–7 days) bands corresponding to $mRNA_{\alpha2}$ became faintly visible but could not be quantified because of the increase in background exposure.

We next examined the expression of Na,K-ATPase subunit mRNAs during neonatal development of euthyroid rats between the second to fifteenth postnatal days (Fig. 2). In the figure, subunit-mRNA abundances at each time point were standardized per unit DNA and, for purposes of comparison, the abundance of each of α_1-, α_3-, and β_1-mRNA on the second postnatal day and that of $mRNA_{\alpha2}$ on the 15th postnatal day were set to 1.0. The abundance of $mRNA_{\beta1}$ did not change significantly during the 13-day interval. $mRNA_{\alpha1}$ abundance decreased to a value of 0.65 by day 15, but the change was not significant (P = 0.10). $mRNA_{\alpha2}$ became measurable on day 5 and increased in abundance such that by day 15 $mRNA_{\alpha2}/mRNA_{\alpha1}$ ratio was 0.25. In contrast to $mRNA_{\alpha2}$, $mRNA_{\alpha3}$ abundance was significantly

Table 2. Effect of T_3 on the RNA, DNA, and protein content of neonatal rat myocardium between the second and fourth postnatal days

Age (day)	T_3	RNA (mg/g)	DNA (mg/g)	Protein (mg/g)	RNA/DNA (mg/mg)
2	–	4.5 ± 0.5	6.7 ± 0.7	125 ± 9	0.68 ± .03
3	–	3.8 ± 0.1	5.2 ± 0.3	107 ± 3	0.73 ± .03
3	+	4.6 ± 0.4	5.6 ± 0.4	118 ± 4	0.81 ± .06
4	–	4.2 ± 0.2	6.9 ± 0.4	134 ± 6	0.61 ± .02
4	+	5.6 ± 0.6	6.8 ± 0.8	142 ± 10	0.83 ± .06[a]

Beginning on the second postnatal day, rats were treated daily with T_3 (75 μg/100 g body weight) or diluent alone and sacrificed on the postnatal days indicated. Hearts from 3–5 rats were pooled to obtain a single sample for analysis from each of the treatment groups shown. Results of tissue contents are expressed as mg/g wet weight (mean ± SE; n = 4).
[a] Denotes $P < 0.05$.

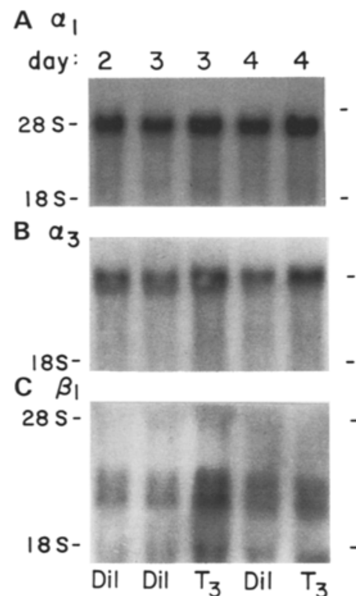

Fig. 3. Effect of T_3 on the expression of myocardial Na,K-ATPase mRNA subunit mRNAs between the second to fourth postnatal days. Litters were randomly assigned to two treatment groups and the neonates received either T_3 (75 μg/100 g body weight) or diluent alone on postnatal days 2 and 3 and were studied on postnatal days 3 and 4. RNA was isolated from 4–6 pooled hearts, fractionated, and probed with the Na, K-ATPase subunit cDNAs as described in the legend to Fig. 1. Panels *A, B,* and *C* show the results of the corresponding blots probed with $cDNA_{\alpha1}$, $cDNA_{\alpha3}$, and $cDNA_{\beta1}$, respectively

decreased on day 10 and reached ~15% of its day-2 value by the 15th postnatal day. These data are consistent with the results reported previously by Orlowski and Lingrel [29], and demonstrate that α_1-, α_3-, and β_1-mRNA abundances are virtually constant during the period between the second to the fifth postnatal days, and that $mRNA_{\alpha2}$ becomes detectable by the fifth postnatal day.

To examine whether T_3 increases the abundance of Na,K-ATPase subunit mRNAs and elicits a premature "switching" of the Na,K-ATPase $mRNA_\alpha$ expression in the myocardium, the effect of T_3 on the abundance of subunit mRNAs was determined between the second and fourth postnatal days. Because of potential changes in tissue RNA content resulting from T_3 treatment [2, 10, 11], we first measured myocardial protein, RNA, and DNA content in diluent- and T_3-treated neonates (Table 2). T_3 increased the RNA content and the RNA/DNA ratio of the myocardium on the third and fourth postnatal days, but only the change in RNA/DNA ratio on day 4 was statistically significant. The effect of T_3 on Na,K-ATPase subunit-mRNA abundances is shown in Fig. 3. The abundances of $mRNA_{\alpha1}$, $mRNA_{\alpha3}$, and $mRNA_{\beta1}$ appeared to be increased by T_3. This experiment was repeated four times and the averaged results are shown in Fig. 4. In the figure, all values have been standardized per unit DNA based upon the values listed in Table 2. The abundance of the three mRNAs remained unchanged in the diluent-treated neonates, in accordance to the results of Fig. 2. In contrast, T_3 increased the abundance of the three subunit mRNAs significantly both at 24 and 48 hr. The abundance of $mRNA_{\beta1}$ showed a larger increment than that of the α-subunit mRNAs at 24 hr, and its abundance then declined to reach a net increment comparable to the increments in α_1- and α_3-mRNAs by 48 hr. T_3 did not result in a premature disappearance $mRNA_{\alpha3}$, and indeed $mRNA_{\alpha3}$ abundance increased following treatment with the hormone. $mRNA_{\alpha2}$ abundance was too low to be accurately measured during the 48-hr interval and treatment with T_3 did not appear to induce a premature expression of the $mRNA_{\alpha2}$ isoform.

Discussion

The developmental changes in rat myocardial Na,K-ATPase mRNA abundances shown in Figs. 1 and 2 are consistent with the results reported by Orlowski and Lingrel [29] and demonstrate that, during the short interval between the second to the fifth postnatal days, the abundances of $mRNA_{\alpha1}$, $mRNA_{\alpha3}$, and $mRNA_{\beta}$ remain relatively constant. The results furthermore show that the abundance of $mRNA_{\alpha3}$

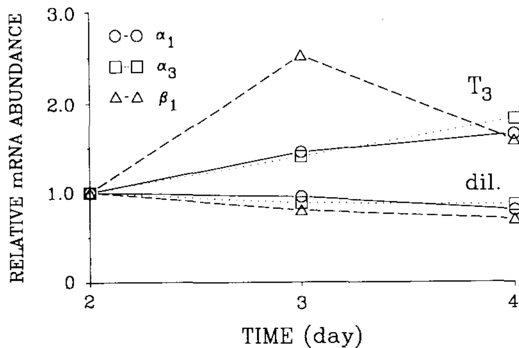

Fig. 4. Effect on T_3 on the relative abundance of Na,K-ATPase subunit mRNAs between the second to fourth postnatal days. Conditions were as described in the legend to Fig. 3. The experiment was repeated four times and the results averaged. To express the relative abundances of the mRNAs per unit DNA at each of the time points and for each treatment condition, the respective abundances were multiplied by the RNA/DNA ratios listed in Table 2. The resulting mRNA abundances were then normalized to their corresponding day-2 values. None of the changes in the mRNA abundances in the diluent-treated group were significant. In contrast, all the increments in the T_3-treated group on days 3 and 4 were significant. The abundance of $mRNA_{\beta 1}$ in T_3-treated neonates on day 3 was significantly larger than its value on day 4

markedly decreases and that of $mRNA_{\alpha 2}$ increases between the 5th and 15th postnatal days, as reported previously [29].

Previous studies have demonstrated that thyroid hormone concentrations in the serum of neonatal rats are negligible at birth and increase dramatically during the first two weeks postnatally [6, 7, 45]. Furthermore, thyroid hormones are known to augment the activity of Na,K-ATPase in target tissues including myocardium, an enhancement that is preceded by increases in the abundances of the subunit mRNAs that in turn are mediated in part at the transcriptional level [2, 10, 11, 27]. We thus hypothesized that T_3 may act as a "molecular switch" during the early postnatal development of myocardial Na,K-ATPase expression. We elected to examine the effect of T_3 on the expression of Na,K-ATPase subunit mRNAs between days 2 and 4 postnatally, with the prediction that if T_3 action is instrumental in the $mRNA_{\alpha}$-isoform switching, then a premature expression of $mRNA_{\alpha 2}$ and disappearance of $mRNA_{\alpha 3}$ should be observed following T_3 treatment. In experiments designed to test this hypothesis we found that T_3 treatment increased the abundances of α_1-, α_3-, and β_1-mRNAs in the myocardium, demonstrating that Na,K-ATPase subunit-mRNA expression in the neonatal rat heart is regulated by T_3 (Figs. 3 and 4). Treatment with T_3 did not result in a premature induction of the $mRNA_{\alpha 2}$ isoform. The abundance of $mRNA_{\alpha 3}$, however, increased significantly with T_3, a finding that has not been described previously. These results suggest that the hormone does not act as a "molecular switch" in the respective "up"- and "down"-regulation of myocardial Na, K-ATPase $mRNA_{\alpha 2}$ and $mRNA_{\alpha 3}$ expression. Instead the findings demonstrate that T_3 enhances the expression of the Na,K-ATPase subunit genes that are already being expressed in this tissue at the time of treatment and suggest that the switching between myocardial α_2- and α_3-mRNA isoforms is regulated by mechanisms other than thyroid hormone action. The above inference is consistent with the lack of $mRNA_{\alpha 3}$ induction in myocardium of adult hypothyroid rats treated with "receptor-saturating" doses of T_3 [11, 28]. It should be noted that in the present study the effect of T_3 was examined during the interval between the second and fourth postnatal days. Thus the possibility that the hormone may still play an important role in Na,K-ATPase $mRNA_{\alpha}$ switching at later developmental stages cannot be excluded. In a recent study Orlowski and Lingrel [30] examined the effects of T_3 and dexamethasone on the expression of Na,K-ATPase $mRNA_{\alpha}$ isoforms in rat neonatal cardiac myocytes in culture. They too found that the addition of T_3 in this *in-vitro* system did not elicit a "switching" between the $mRNA_{\alpha}$ isoforms. However, a complex relationship between $mRNA_{\alpha}$-isoform expression following the addition of dexamethasone was noted, suggesting a potential role of this latter hormone during myocardial development [30].

The results of the present study demonstrating a lack of T_3 effect on "switching" of Na,K-ATPase $mRNA_{\alpha}$ isoforms should be contrasted to previous results demonstrating that T_3 acts as a "molecular switch" in the regulation of skeletal muscle and cardiocyte myosin heavy chain expression [12, 13, 19, 20, 33]. This difference might reflect the influence of specific regulatory mechanisms operative in the control of myosin heavy chain and Na,K-ATPase gene expression. Consistent with the above interpretation is the presence of "thyroid response elements" upstream to the α- and β-myosin heavy chain genes and the lack of such an element in the 5′-upstream flanking region of the human Na,K-ATPase α_2 gene [12, 13, 19, 37]. Although it is not known whether "thyroid response elements" are present upstream in the other Na,K-ATPase subunit genes, and T_3 regulatory sites may be located at sites other than 5′-upstream to the α_2 gene, the above findings raise the possibility of indirect regulation of Na,K-ATPase gene expression by T_3. Indeed, we have noted that thyroidal enhancement of $mRNA_{\beta 1}$ abundance in Clone 9 cells is abrogated by prior exposure to cycloheximide, while ongoing protein

synthesis is not required for the induction of $mRNA_{\alpha 1}$ by the hormone [9]. Alternatively, it should be noted that adrenergic innervation and catecholamine uptake capacity of sympathetic neurons in neonatal rat myocardium is poorly developed at birth and matures during the first several days of postnatal development [5, 31]. It is thus possible that events associated with adrenergic neuronal activity may influence the pattern of myocardial Na,K-ATPase $mRNA_{\alpha}$-isoform expression. Further studies are thus required to elucidate mechanisms underlying the molecular "switching" of myocardial Na,K-ATPase expression, and to explore the basis of responsiveness of these genes to T_3 at various stages of the developmental program.

We thank Drs. G.E. Shull and J.B. Lingrel for their generous gift of full-length Na,K-ATPase α_1-, α_2-, α_3-, and β_1-subunit cDNAs. This study was supported by National Institutes of Health Grant HL-39300.

References

1. Charlemagne, D., Mayoux, E., Poyard, M., Oliviero, P., Geering, K. 1987. Identification of two isoforms of the catalytic subunit of Na,K-ATPase in myocytes isolated from adult rat heart. *J. Biol. Chem.* **262**:8941–8943
2. Chaudhury, S., Ismail-Beigi, F., Gick, G.G., Levenson, R., Edelman, I.S. 1987. Effect of thyroid hormone on the abundance of Na,K-adenosine triphosphatase α-subunit messenger ribonucleic acid. *Mol. Endocrinol.* **1**:83–89
3. Chirgwin, J.M., Przbyla, A.E., MacDonald, R.J., Rutter, W.J. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**:5294–5299
4. Curfman, G.D., Crowley, T.J., Smith, T.W. 1977. Thyroid induced alterations in myocardial sodium-and potassium-activated adenosine triphosphatase, monovalent cation active transport, and cardiac glycoside binding. *J. Clin. Invest.* **59**:586–590
5. Drugge, E.D., Rosen, M.R., Robinson, R.B. 1985. Neuronal regulation of the development of the α-adrenergic chronotropic response in the rat heart. *Circ. Res.* **57**:415–423
6. Dubois, J.D., Dussault, J.H. 1977. Ontogenesis of thyroid function in the neonatal rat. Thyroxine (T_4) and triiodothyronine (T_3) production rates. *Endocrinology* **101**:435441
7. Dussault, J.H., Labrie, F. 1975. Development of hypothalamic-pituitary-thyroid axis in the neonatal rat. *Endocrinology* **97**:1321–1324
8. Emanuel, J.R., Garetz, S., Stone, L., Levenson, R. 1987. Differential expression of Na,K-ATPase α and β subunit mRNA's in rat tissues and cell lines. *Proc. Natl. Acad. Sci. USA* **84**:9030–9034
9. Gick, G.G., Ismail-Beigi, F. 1990. Thyroid hormone induction of Na,K-ATPase and its mRNAs in a rat liver cell line. *Am. J. Physiol.* **258**:C544–C551
10. Gick, G.G., Ismail-Beigi, F., Edelman, I.S. 1988. Thyroidal regulation of rat renal and hepatic Na,K-ATPase gene expression. *J. Biol. Chem.* **263**:16610–16618
11. Gick, G.G., Melikian, J., Ismail-Beigi, F. 1990. Thyroidal enhancement of rat myocardial Na,K-ATPase: Preferential expression of $\alpha 2$ activity and mRNA abundance. *J. Membrane Biol.* **115**:273–282
12. Gustafson, T.A., Bahl, J.J., Markham, B.E., Roeske, W.R., Morkin, E. 1987. Hormonal regulation of myosin heavy chain and α-actin gene expression in cultured fetal rat heart myocytes. *J. Biol. Chem.* **262**:13316–13322
13. Gustafson, T.A., Markham, B.E., Bahl, J.J., Morkin, E. 1987. Thyroid hormone regulates expression of a transfected α-myosin heavy chain fusion gene in fetal heart cells. *Proc. Natl. Acad. Sci. USA* **84**:3122–3126
14. Haber, R.S., Loeb, J.N. 1988. Selective induction of high ouabain-affinity isoform of Na^+-K^+-ATPase by thyroid hormone. *Am. J. Physiol.* **255**:E912–E919
15. Hara, Y., Nikamato, A., Kojima, T., Matsumoto, A., Nakao, M. 1988. Expression of sodium pump activities in BALB/c 3T3 cells transfected with cDNA encoding the α_3-subunits of rat brain Na^+, K^+-ATPase. *FEBS Lett.* **238**:27–30
16. Hubert, J.J., Schenk, D.B., Skelly, H., Leffert, H.L. 1986. Rat hepatic (Na^+,K^+)-ATPase α-subunit isolation by immunoaffinity chromatography and structural analysis by peptide mapping. *Biochemistry* **25**:4156–4163
17. Ismail-Beigi, F., Edelman, I.S. 1971. The mechanism of the calorigenic action of thyroid hormone. *J. Gen. Physiol.* **57**:710–722
18. Ismail-Beigi, F., Edelman, I.S., Gick, G.G. 1988. Greater induction of Na,K-ATPase alpha-2 *versus* alpha-1 mRNA abundance by thyroid hormone in rat skeletal muscle and ventricular myocardium. *Clin. Res.*. **36**:542 (*Abstr.*)
19. Izumo, S., Mahdavi, V. 1988. Thyroid hormone receptor α isoforms generated by alternative splicing differentially activate myosin HC gene transcription. *Nature (London)* **334**:539–542
20. Izumo, S., Nadal-Ginard, B., Mahdavi, V. 1986. All members of the myosin heavy chain multi-gene family respond to thyroid hormone in a highly tissue specific manner. *Science* **231**:597–600
21. Jorgensen, P.L. 1982. Mechanism of the Na^+,K^+ pump. Protein structure and conformations of the pure ($Na^+ + K^+$)-ATPase. *Biochim. Biophys. Acta* **694**:27–68
22. Lin, M.H., Akera, T. 1978. Increased ($Na^+ + K^+$)-ATPase concentrations in various tissues of rats caused by thyroid hormone treatment. *J. Biol. Chem.* **253**:723–726
23. Lo, C.-S., Edelman, I.S. 1976. The effect of triiodothyronine on the synthesis and degradation of renal cortical ($Na^+ + K^+$)-adenosine triphosphatase. *J. Biol. Chem.* **251**:7834–7840
24. Lo, C.-S., Lo, T.N. 1980. Effect of triiodothyronine on the synthesis and degradation of the small subunit of renal cortical ($Na^+ + K^+$)-adenosine triphosphatase. *J. Biol. Chem.* **225**:2131–2136
25. Lytton, J., Lin, J.C., Guidotti, G. 1985. Identification of two molecular forms of (Na^+,K^+)-ATPase in rat adipocytes. *J. Biol. Chem.* **260**:1177–1184
26. Martin-Vasallo, P., Dackowski, W., Emanuel, J.R., Levenson, R. 1989. Identification of a putative isoform of the Na,K-ATPase β subunit. Primary structure and tissue-specific expression. *J. Biol. Chem.* **264**:4613–4618
27. McDonough, A.A., Brown, T.A., Horowitz, B., Chiu, R., Schlotterbeck, J., Bowen, J., Schmitt, C.A. 1988. Thyroid hormone coordinately regulates $Na^+ - K^+$-ATPase α- and β-subunit mRNA levels in the kidney. *Am. J. Physiol.* **254**:C323–C329
28. Oppenheimer, H.H. 1983. The nuclear receptor-triiodothyronine complex: Relationship to thyroid hormone distribution,

metabolism, and biological action. *In:* Molecular Basis of Thyroid Hormone Action. J.H. Oppenheimer and H.H. Samuels, editors. pp. 1–34. Academic, New York

29. Orlowski, J., Lingrel, J.B. 1988. Tissue-specific and developmental regulation of rat Na,K-ATPase catalytic α isoform and β subunit mRNAs. *J. Biol. Chem.* **263:**10436–10442
30. Orlowski, J., Lingrel, J.B. 1990. Thyroid and glucocorticoid hormone regulate the expression of multiple Na,K-ATPase gene in cultured neonatal rat cardiac myocyte. *J. Biol. Chem.* **265:**3462–3470
31. Pappano, A.J. 1977. Ontogenetic development of autonomic neuroeffector transmission and transmitter reactivity in embryonic and fetal hearts. *Pharmacol. Rev.* **29:**3–33
32. Philipson, K.D., Edelman, I.S. 1977. Thyroid hormone control of $Na^+ + K^+$-ATPase and K^+-dependent phosphatase. *Am. J. Physiol.* **232:**C196–C201
33. Russell, S.D., Cambon, N., Nadal-Ginard, B., Whalen, R.G. 1988. Thyroid hormone induces a nerve-independent precocious expression of fast myosin heavy chain mRNA in rat hindlimb skeletal muscle. *J. Biol. Chem..* **263:**6370–6374
34. Schneider, J.W., Mercer, R.W., Gilmore-Herbert, M., Utset, M.F., Lai, C., Greene, A., Benz, E.D., Jr. 1988. Tissue specificity, localization in brain, and cell-free translation of mRNA encoding the A3 isoform of Na^+,K^+-ATPase. *Proc. Natl. Acad. Sci. USA* **85:**284–288
35. Shull, G.E., Greeb, J., Lingrel, J.B. 1986. Molecular cloning of three distinct forms of the Na^+,K^+-ATPase α-subunit form from brain. *Biochemistry* **25:**8125–8132
36. Shull, G.E., Lane, L.K., Lingrel, J.B. 1986. Amino-acid sequence of the β subunit of the $(Na^+ + K^+)$ATPase deduced from a cDNA. *Nature (London)* **321:**429–431
37. Shull, M.M., Pugh, D.G., Lingrel, J. 1989. Characterization of the human Na,K-ATPase $\alpha 2$ gene and identification of intragenic restriction fragment length polymorphism. *J. Biol. Chem.* **264:**17535–17543
38. Shyjan, A.W., Gottardi, C., Levenson, R. 1990. The Na,K-ATPase $\beta 2$ subunit is expressed in rat brain and copurifies with Na,K-ATPase activity. *J. Biol. Chem.* **265:**5166–5169
39. Snedecor, G.W., Cochran, W.G. 1976. Statistical Methods. Iowa University Press, Ames (IA)
40. Sweadner, K.J. 1979. Two molecular forms of $(Na^+ + K^+)$-stimulated ATPase in brain. *J. Biol. Chem.* **254:**6060–6067
41. Sweadner, K.J., Farshi, S.K. 1987. Rat cardiac ventricle has two Na^+,K^+-ATPases with different affinities for ouabain: Developmental changes in immunologically different catalytic subunits. *Proc. Natl. Acad. Sci. USA* **84:**8404–8407
42. Thomas, P.S. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA* **77:**5201–5205
43. Urayama, O., Shutt, H., Sweadner, K.J. 1989. Identification of three isozyme proteins of the catalytic subunit of the Na,K-ATPase in rat brain. *J. Biol. Chem.* **264:**8271–8280
44. Urayama, O., Sweadner, K.J. 1988. Ouabain sensitivity of alpha 3 isozyme of rat Na,K-ATPase. *Biochem. Biophys. Res. Commun.* **156:**796–800
45. Young, R.A., Meyers, B., Alex, S., Fang, S., Braverman, L.E. 1988. Thyroxine binding to serum thyronine-binding globulin in thyroidectomized adult and normal neonatal rats. *Endocrinology* **122:**2318–2323
46. Young, R.M., Lingrel, J.B. 1987. Tissue distribution of mRNAs encoding the alpha isoforms and beta subunit of rat Na^+,K^+ATPase. *Biochem. Biophys. Res. Commun.* **145:**52–58
47. Yuh, Y.-S., Thompson, E.B. 1989. Glucocorticoid effect on oncogene/growth gene expression in human T lymphoblastic leukemic cell line CCRF-CEM. Specific c-*myc* mRNA suppression by dexamethasone. *J. Biol. Chem.* **264:**10904–10910

Received 30 April 1990; revised 17 July 1990