Neonatal hyper- and hypothyroidism alter the myoglobin gene expression program in adulthood

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Abstract

Myoglobin acts as an oxygen store and a reactive oxygen species acceptor in muscles. We examined myoglobin mRNA in rat cardiac ventricle and skeletal muscles during the first 42 days of life and the impact of transient neonatal hypo- and hyperthyroidism on the myoglobin gene expression pattern. Cardiac ventricle and skeletal muscles of Wistar rats at 7-42 days of life were quickly removed, and myoglobin mRNA was determined by Northern blot analysis. Rats were treated with propylthiouracil (5-10 mg/100 g) and triiodothyronine (0.5-50 µg/100 g) for 5, 15, or 30 days after birth to induce hypo- and hyperthyroidism and euthanized either just after treatment or at 90 days. During postnatal (P) days 7-28, the ventricle myoglobin mRNA remained unchanged, but it gradually increased in skeletal muscle (12-fold). Triiodothyronine treatment, from days P0-P5, increased the skeletal muscle myoglobin mRNA 1.5- to 4.5-fold; a 2.5-fold increase was observed in ventricle muscle, but only when triiodothyronine treatment was extended to day P15. Conversely, hypothyroidism at P5 markedly decreased (60%) ventricular myoglobin mRNA. Moreover, transient hyperthyroidism in the neonatal period increased ventricle myoglobin mRNA (2-fold), and decreased heart rate (5%), fast muscle myoglobin mRNA (30%) and body weight (20%) in adulthood. Transient hypothyroidism in the neonatal period also permanently decreased fast muscle myoglobin mRNA (30%) and body weight (14%). These results indicated that changes in triiodothyronine supply in the neonatal period alter the myoglobin expression program in ventricle and skeletal muscle, leading to specific physiological repercussions and alterations in other parameters in adulthood.

Key words: Muscle; Thyroid hormone; Long-term consequences; Early postnatal life

Introduction

Myoglobin (Mb) is an important intracellular oxygen (O_2) binding protein that is highly expressed in cardiac and skeletal muscles (1), and highly conserved among species (2). The oxymyoglobin structure reveals its role as a muscular store of O_2 (3); additionally, this protein functions as a scavenger of reactive species of O_2 and nitric oxide (4). Although Mb plays an essential role in oxidative metabolism, Mb-knockout mice (myo-/-) survive as a consequence of several adjustments in cardiac metabolism (5). Increased capillary density, coronary blood flow, and elevated hematocrit combine to assure an increase in the PO_2 gradient in mitochondria, similar to effects that occur in response to the hypoxia observed in exercise and pregnancy (6,7).

Thyroid hormone (TH) was shown to upregulate Mb gene expression in adult rats (8,9), but, there is scarce information on this effect in the postnatal period, particularly in the first month of life, when skeletal and cardiac muscles are not yet completely mature. In 1996, Garry et al. (10) demonstrated a progressive enhancement of Mb expression following birth in mice, which paralleled the expression of several proteins associated with the phenotype of mature skeletal muscle. On the other hand, the pattern of Mb gene expression in cardiac muscle in the period of early postnatal development has not yet been examined and deserves to be evaluated.

Cardiac and skeletal muscles are still immature at birth. Their postnatal development includes the transition of the expression pattern of many genes and replacement of fetal contractile and metabolic proteins to the adult isoforms (11-13). TH plays an important role in the establishment of these changes, some of which occur in parallel with the increase in serum TH concentration normally observed during that period (14).

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In adult life, the major effects of hypo- and hyperthyroidism on skeletal and cardiac muscles are abolished when the subjects return to the euthyroid state. However, in the early postnatal period, when the pattern of expression of many genes is being established, alterations in thyroid function can lead to modifications in the gene expression programs of adulthood (15).

In this study, we evaluated Mb mRNA expression in rat cardiac and skeletal muscles during postnatal development and after induction of hyper- and hypothyroidism in the first 2 weeks of life. We also investigated the effects of inducing hypo- and hyperthyroidism from postnatal days zero (P0) to 30 (P30) on Mb gene expression in heart ventricle and skeletal muscles, ventricle weight, heart rate (HR) and arterial blood pressure (ABP) in adulthood.

Material and Methods

Animals and treatments

Animal care and experimental procedures performed in this study followed the ethical principles for animal research adopted by Brazil's National Council of Animal Experimentation (CONCEA) and were approved by the Ethics Committee for Animal Research (CEEA) of the Instituto de Ciências Biomédicas, Universidade de São Paulo.

Pregnant Wistar rats were obtained from the breeding colony of the Instituto de Ciências Biomédicas. They had free access to commercial chow and tap water in a temperature-controlled room ($23 \pm 1°C$) with a 12-h light-dark cycle (lights on at 7:00 am). Newborn male Wistar rats weighing approximately 6 g were used in the study. In each group, treatment began on the first postnatal day (P1) after birth (P0). Each litter consisted of approximately eight rats per dam, and animals were maintained under controlled conditions as described above from days P1 through P42. Rats were randomly assigned to the following three experiments: *i*) Mb gene expression in heart ventricle and skeletal muscles was determined during postnatal development on days P7, P14, P21, P28, P35, and P42 in groups consisting of 12 rats each. *ii*) The effect of TH on the expression of Mb mRNA in heart ventricle and skeletal muscles was determined during the first 2 weeks of life. Newborn rats were assigned to groups of 6 rats each for subcutaneous (*sc*) injection of 0.5, 1.0, 1.5, 2.0, 4.0, or 50.0 µg/100 g body weight (BW) triiodothyronine (T_3; Sigma, USA; Hyper-5 group), groups of 12 rats each for *sc* injection of 5 or 10 mg/100 g BW propylthiouracil (PTU; Sigma; Hypo-5 group), or saline (0.9% NaCl; Control-5 group), for 5 days beginning on P1. Other groups of 12 newborn rats each were treated with *sc* injection of 4 µg T_3/100 g BW (Hyper-15 group), 5 mg PTU/100 g BW (Hypo-15 group), or saline (Control-15 group), for 15 days beginning on P1. The lactating dams of the Hypo-5 and Hypo-15 groups were also treated with PTU (5 mg/100 g BW) during the lactation period, to avoid T_3 transfer to pups

through milk. *iii*) Mb gene expression programming in heart ventricle and skeletal muscles was evaluated after induction of transient postnatal hyperthyroidism (HyperPNT) or hypothyroidism (HypoPNT). Newborn rats were exposed to T_3 (4 µg/100 g BW), PTU (5 mg/100 g BW), or saline by *sc* injection for 30 days beginning at birth (P1). After this period, the treatments were stopped, and the animals were followed until they were 90 days old. They constituted the HyperPNT and HypoPNT groups. These animals were anesthetized, killed, and the repercussions of HyperPNT or HypoPNT induction on Mb gene expression, ventricular/body weight ratio, HR and ABP were evaluated. Euthyroid animals were used as controls (Control-90 group). Each group consisted of 12 rats.

For the hormonal treatments, 100 µg T_3 was dissolved in 2 N NaOH (~20 µL), diluted with 0.9% NaCl to reach the concentrations used in the study, and adjusted to pH 7.4. The animals were fasted for 2 h, anesthetized with ketamine and xylazine (100 and 10 mg/kg BW, respectively) and killed by decapitation, after the specified periods of treatment. Handling of the offspring during the treatment period was performed by the same person throughout the experiment. A heat lamp was used to avoid cool-down.

Heart ventricle and skeletal muscles were quickly removed under aseptic conditions, for total RNA extraction as described previously (8). In experiments I and II, the skeletal muscle sample used was representative of the whole distal hind limb muscle, whereas in experiment III, the fast extensor digitalis longus (EDL) and slow soleus muscles were used.

To check the efficiency of the T_3 and PTU treatments, heart ventricle weight and the day on which pups opened their eyes (DEO) were determined. Blood samples were also collected to evaluate thyroxine (T_4) serum concentrations by radioimmunoassay (RIA; Diagnostic Products Corporation, UK), using a standard curve prepared with different concentrations of T_4 in TH-free rat serum. Intra- and interassay coefficients of variation for the T_4 RIA were 3-10%, and 4.6-10%, and 5.0-6.5% and 4.2-6.0%, for the T_3 RIA, respectively.

The most effective T_3 dose in inducing Mb gene expression in both tissues was 4 µg/100 g BW, a dose 13-times higher than the physiological replacement dose of T_3, which is postulated to be 0.3 µg/100 g BW (15,16), but lower than those used in similar studies (17). The PTU dose chosen to evaluate the effects of long-term hypothyroidism (15 and 30 day-treatment) was 5 mg/100 g BW, a dose equivalent to half of those used in comparable studies (11,18).

Total RNA extraction and Northern blot analysis

Total RNA was isolated using the acid guanidinium thiocyanate-phenol-chloroform extraction method and quantified by absorbance at 260 nm. Six micrograms of total RNA samples were denatured with formaldehyde-formamide, electrophoresed in 1% agarose gels containing

2.2 M formaldehyde in $1 \times$ 3-n-morpholino-propanesulfonic acid (MOPS) buffer and blotted onto a nylon membrane (Nylon-1 membrane, Gibco BRL, USA) by neutral capillary transfer. The cross-linking of the RNA to the membrane was carried out at 80°C for 2 h in a vacuum oven, and prehybridized in 50% formamide hybridization solution and 100 µg/mL denatured salmon sperm DNA at 42°C for 4 h. Subsequently, the membrane was probed with a ^{32}P-labeled rat Mb cDNA by random priming (Random Primers DNA Labeling System kit, Gibco BRL) for 16 h at 42°C. The membrane was washed under high stringency conditions and subjected to autoradiography and quantified by phosphor imaging, using the ImageQuant software (Molecular Dynamics, USA). All blots were stripped and rehybridized with a ^{32}P-labeled RNA probe specific for the 18s ribosomal subunit (18s rRNA), synthesized by *in vitro* transcription (Maxi Script *in vitro* transcription kit, Ambion, USA), to correct for the variability in RNA loading. The results are reported as means ± SE of Mb mRNA/18S rRNA ratio.

HR and ABP determination

Animals in the Control-90, HyperPNT, and HypoPNT groups were maintained in a chamber at 45°C for 5 min. After this period, they were restrained in acrylic cylinders, and their HR and systolic arterial pressure (SBP) were measured with a tail-cuff phethysmograph, an indirect method for measuring blood pressure (Kent Scientific USA). Data were collected after the animals had been subjected to the same procedure 3 times, at approximately the same time of day, in the previous weeks.

Statistical analysis

The results were analyzed using one-way ANOVA, followed by the Student-Newman-Keuls test. Data are reported as means ± SE and were analyzed using GraphPad PRISM, version 3.0 (GraphPad Software, USA). Differences with P values ≤0.05 were considered to be statistically significant.

Results

The effectiveness of the T_3 and PTU treatments was evaluated by determining the ratio of ventricular weight to body weight (VW/BW), DEO, and serum T_4 concentration. Rats treated with T_3 (4 µg/100 g BW, *sc*) for 5, 15, and 30 days after P1 presented an increase in the VW/BW ratio, an earlier DEO, and decreased T_4 levels, as expected. Rats treated with PTU (5 and 10 mg/100 g BW) showed a delay in the DEO and decreased serum T_4 levels. These results are presented in Table 1 and confirmed the expected effects.

Experiment I: evaluation of Mb gene expression in heart ventricle and skeletal muscles during rat postnatal development

The pattern of Mb mRNA expression in heart ventricle

and skeletal muscles of rats, from P7 through P42, is illustrated in Figure 1. Ventricle Mb mRNA content remained constant during the first 4 weeks of life, except on day P21, when a transitory increase was detected; then, a progressive increase in ventricular Mb mRNA expression occurred from days P35 to P42 (Figure 1A). In contrast, in the distal hind limb muscles, a gradual increase of Mb mRNA expression was observed from days P7 to P28, and remained unchanged up to day P42, when the Mb mRNA achieved a 12-times higher value than that observed at day P7 (Figure 1B).

Experiment II: TH effect on heart ventricle and skeletal muscle Mb mRNA expression during the first 2 weeks of life

Responsiveness of the Mb gene to TH was evaluated by determining Mb mRNA content in ventricle and skeletal muscles of rats treated for 5 days with increasing doses of T_3 (0.5, 1.0, 1.5, 2.0, 4.0, and 50 µg/100 g BW; dose-response curve). Data from this experiment are summarized in Figure 2. No alteration in the ventricle muscle Mb mRNA expression was observed, even with the highest T_3 dose used (Figure 2A). However, extending T_3 treatment (4 µg/100 g BW) to 15 days effectively increased the ventricle muscle Mb mRNA content (Figure 2C). The 5- and 15-day PTU treatment induced decreases in the ventricle muscle Mb mRNA content of 60% and 20%, respectively (Figure 2B and C).

In contrast to heart ventricle muscle, the administration of increasing doses of T_3 for 5 days raised the Mb mRNA expression in the skeletal muscle, which peaked at a T_3 dose of 4 µg/100 g BW (Figure 2D). At the highest T_3 dose tested (50 µg/100 g BW), a fall in the Mb mRNA expression was observed, even though the values obtained were still higher than those of the control group. Surprisingly, the 5-day PTU treatment with 5 mg/100 g BW led to an increase of Mb mRNA content in skeletal muscles (Figure 2E), followed by a decrease to control values when extending the treatment to day P15 (Figure 2F). When the PTU dose was increased 2-fold, the Mb mRNA content in the 5-day PTU treated rats decreased to the values seen in the control group (Figure 2E). In spite of that, both PTU doses (5 and 10 mg/100 g BW) were able to induce hypothyroidism (Table 1).

Experiment III: reprogramming of Mb gene expression in heart ventricle and skeletal muscles after induction of HyperPNT or HypoPNT

The Mb mRNA content of ventricle, soleus, and EDL muscles from 90 day-old rats in the HyperPNT or HypoPNT groups is summarized in Figure 3. Increased Mb mRNA content was seen in the ventricle muscle of HyperPNT group adults but not in the HypoPNT group adults (Figure 3A). HyperPNT and HypoPNT did not alter soleus muscle Mb mRNA expression in adults (Figure 3B), however both treatments led to a decrease of

Table 1. Effect of thyroid hormone on development of body weight, ventricular weight, VW/BW ratio, DEO, and serum T_4 concentrations of T_3- (4 µg/100 g, *sc*; hyperthyroid group) and PTU- (5 mg/100 g, *sc*; hypothyroid group) treated rats for 5, 15, and 30 days.

Parameters	Control (n = 6)	Hyperthyroid (n = 6)	Hypothyroid (n = 6)
BW (g)			
5 days	15 ± 1	14 ± 1	14 ± 1
15 days	24 ± 1	23 ± 2	25 ± 1
30 days	87 ± 3	72 ± 4#	35 ± 3◆
VW (mg)			
5 days	90 ± 4	161 ± 8◆	83 ± 2
15 days	107 ± 10	152 ± 23*	83 ± 7
30 days	310 ± 20	480 ± 20◆	121 ± 16◆
VW/BW (mg/g)			
5 days	6.1 ± 0.3	11.8 ± 0.3◆	6.1 ± 0.2
15 days	4.4 ± 0.3	6.8 ± 0.5+	3.3 ± 0.2*
30 days	3.6 ± 0.1	6.7 ± 0.3+	3.4 ± 0.1
T_4 (ng/mL)			
5 days	28 ± 1	14 ± 1+	22 ± 3*
15 days	66 ± 3	15 ± 1+	14 ± 1+
30 days	40 ± 1	14 ± 2+	7 ± 1+
DEO (postnatal days)			
5 days	–	–	–
15 days	13	11	14
30 days	13	11	14

Rats treated with vehicle (0.9% NaCl) were used as control. Data are reported as means ± SE. BW: body weight; VW: ventricular weight; DEO: day of eye opening; T_4: thyroxine; T_3: triiodothyronine; PTU: propylthiouracil. *$P < 0.05$, #P 0.01, +$P < 0.001$ compared with control group (ANOVA).

expression in the EDL muscle (Figure 3C).

HyperPNT and HypoPNT effects on body and ventricular weight, serum T3 levels, HR and ABP in adulthood

The results from adult rats (day P90) in the HyperPNT and HypoPNT groups are summarized in Table 2. The only parameter affected by HypoPNT was a decrease in body weight. However, rats in the HyperPNT group showed reduced BW and VW, and a decreased HR in

adulthood, whereas the ABP was not altered. Serum T_3 levels in both groups remained similar to control rats, indicating that the morphological and functional alterations observed in the HyperPNT and HypoPNT group animals resulted from the impact of the experimental treatments carried out in the first 30 days of life.

Discussion

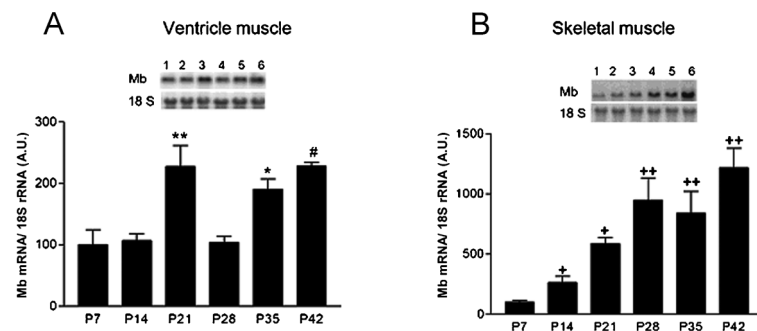In this study, we have shown that: a) Mb gene

Figure 1. Pattern of Mb mRNA expression in ventricular and skeletal muscles of rats throughout the postnatal developmental period. Northern blot analysis of ventricular and skeletal Mb mRNA levels of intact rats from postnatal (P) day 7 through P42 is shown at the top of the figure, and the quantitative results obtained by densitometric analysis of Mb and 18s rRNA transcripts hybridization ratio are shown at the bottom, in arbitrary units (A.U.). Data are reported as means ± SE of 12 animals/group. *$P < 0.05$ and **$P < 0.01$ *vs* P7, P14, and P28; #$P < 0.01$ *vs* all groups except P21; +$P < 0.01$ *vs* all groups; ++$P < 0.05$ *vs* P7, P14, and P21 (one-way ANOVA).
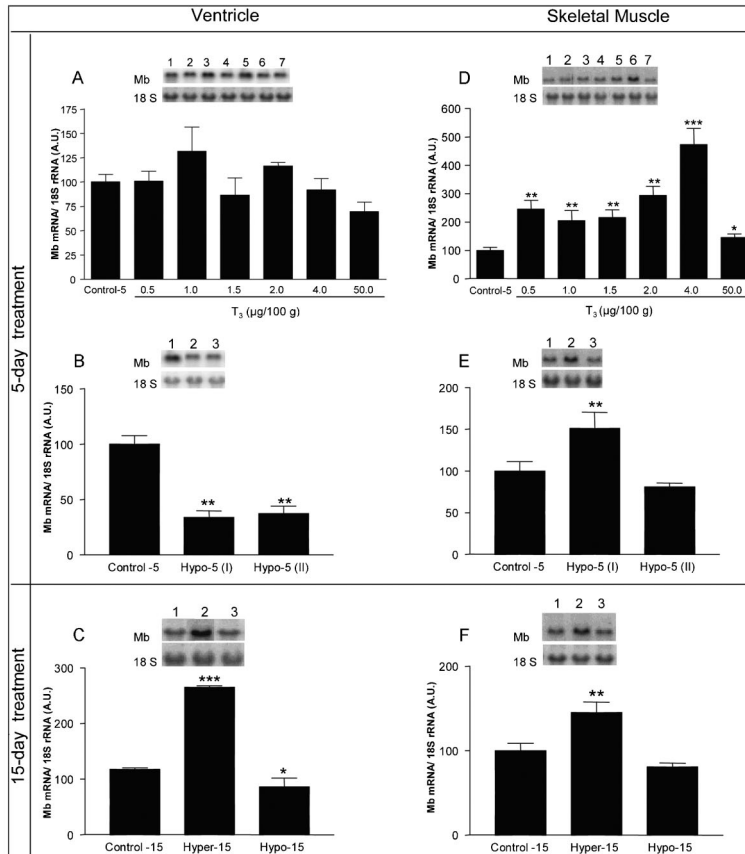
Figure 2. Influence of thyroid hormone on the pattern of Mb gene expression in ventricular and skeletal muscles of rats during 5 and 15 days of postnatal developmental. Northern blot analysis of ventricle and skeletal muscles Mb mRNA levels of rats subjected to T_3 or PTU treatment from P0 through P5 (*A*, *B*, *D*, and *E*) or P15 (*C* and *F*) is shown at the top of each panel, and the quantitative results obtained by densitometric analysis of Mb and 18s rRNA transcripts hybridization ratio are shown at the bottom, in arbitrary units (A.U.). The effect of increasing doses of T_3, from P0 to P5, on Mb mRNA expression is shown in *A* and *D*, and of the PTU treatment [5 mg = Hypo-5 (I), or 10 mg/100 g BW = Hypo-5 (II)] during the same period, is shown in *B* and *E*. Panels *C* and *F* show the effect of T_3 (4 µg/100 g BW) and PTU (5 mg/100 g BW) treatment during the first 15 days of postnatal life on Mb mRNA expression. Data are reported as means ± SE of 12 animals/group. *P<0.05, **P<0.01 and ***P<0.001 *vs* control-5 and *vs* control-15 (one-way ANOVA).

expression differed in heart ventricle and skeletal muscles during the postnatal period, b) TH effects on Mb gene expression were tissue- and age-dependent, c) induction of HyperPNT and HypoPNT altered Mb mRNA levels differently in the cardiac and skeletal muscles of adult rats, and d) transient HyperPNT induced a permanent decrease on VW and HR in adult life.

Compared to skeletal muscle, the heart exhibited a delayed postnatal increase in Mb mRNA expression and required a longer period of time to be affected by hyperthyroidism. The induction of hyperthyroidism led to reprogramming of Mb gene expression as indicated by the increased ventricular Mb mRNA content, and decreased heart rate and ventricle weight in adulthood.

The rat heart starts beating at embryonic day 9.5 (E9.5), whereas the generalized mass movements involving the head, neck, and forelimbs can be seen from E16 onwards. So, considering that muscle activity increases Mb gene expression, one may expect that the heart has a higher Mb mRNA content than skeletal muscle at birth and in the first days of the PN period. This implies that changes of Mb gene expression in the heart could be less evident than those observed in skeletal muscle during this period of development, as observed from the time-course

studies (Figure 1). This observation accounts for the delayed postnatal increase of Mb mRNA expression in the heart when compared with skeletal muscles.

Skeletal muscles achieve the adult phenotype and functional characteristics (19) later than heart muscle. This is the reason that a muscle of the distal hind limb group was used in the time-course experiments and in the studies of Mb mRNA expression in 5- and 15-day-old rats (9). Hence, the Mb expression in skeletal muscle followed its developmental trend. In effect, a 5-fold increase in sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA)1 level has been reported between days P5 and P20 (20), a period in which a significant increase of the Mb gene expression in skeletal muscle (Figure 1B) was also observed. This somehow indicates that muscle fiber differentiation is taking place. In fact, SERCA1 is predominantly expressed in type II (fast), whereas Mb is more abundant in type I (slow) muscle fibers (21).

Cardiac postnatal development is associated with changes in the expression of several genes, some of them coincident with the postnatal increase of TH levels and/or with their peak at the third week of life (22). In the first weeks of life, genes that encode the isoform 1 of the glucose transporter, sodium/calcium exchanger, beta
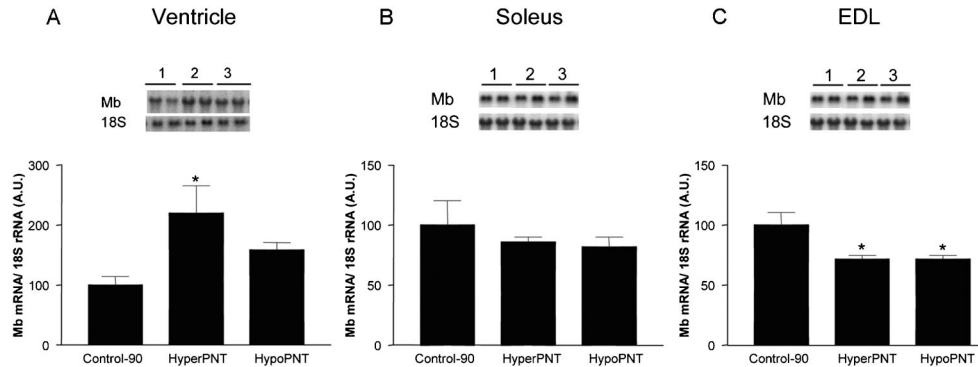
Figure 3. Effect of transient postnatal hyper- and hypothyroidism from postnatal (P) day 0 to P30 on molecular programming of myoglobin gene expression in ventricle (*A*), soleus (*B*) and *extensorum digitalis longus* (EDL) (*C*) muscles of 90-day-old rats. Northern blot analysis of ventricle, soleus, and EDL Mb mRNA levels of rats subjected to vehicle (control-90), T_3 (4 µg/100 g BW, HyperPNT) and PTU (5 mg/100 g BW, HypoPNT) treatment from P0 through P30, and allowed to grow with no further treatment until 90 days of life, is shown at the top of each panel, and the quantitative results obtained by densitometric analysis of Mb and 18s rRNA transcripts hybridization ratio is shown at the bottom, in arbitrary units (A.U). Data are reported as means ± SE of 12 animals/group. *P<0.05 *vs* control-90 (ANOVA).

isoform of the myosin heavy chain and the β1 isoform of the TH receptor have a decreased expression, in contrast to those genes that encode the isoform 4 of the glucose transporter, SERCA2, alpha myosin heavy chain and alpha isoform of thyroid receptor (17,23-25). These molecular changes alter glucose and intracellular calcium availability, the speed of muscle contraction, and there is evidence that the pattern of expression of these genes, which are known targets of TH, can be influenced by the thyroid state (26,27).

In fact, hyperthyroidism leads to a hyperkinetic heart state, which could be deleterious in the first stages of postnatal development (28). However, the heart shows tissue-specific regulation mechanisms for TH action in the postnatal period, such as increased expression of TRα2 (a non-ligand-binding TH receptor isoform) (29) and significant TH inactivation (30), which ensure its protection against the impact of TH in this period, even in the presence of increased TH levels.

The lack of ventricular Mb gene responsiveness to the progressive increase of T_3 doses observed in 5-day-old rats, in contrast to the response of adult rats (9), supports this proposition. This response is similar to that reported for neonatal cardiomyocytes treated with growth hormone (GH), which, unlike adult cardiomyocytes, are refractory to GH effects, because GH receptors are not functional in intrauterine and early postnatal life (31). In contrast, the skeletal muscle of 5-day-old rats exhibited increased Mb mRNA content in response to T_3, an effect that was greatest at 4 µg T_3/100 g BW, a dose 12.5-fold smaller than the highest used in the study. Indeed, high doses of T_3 induce protein catabolism, which might explain the strong response to a low T_3 dose (32).

As no thyroid response element has been shown in the promoter region of the Mb gene, we postulate that tissue-specific factors might be involved in cardiac- and skeletal-muscle responsiveness to this hormone. This strengthens the possibility that the Mb gene may be

Table 2. Body weight, ventricular weight, VW/BW ratio, serum T_3, heart rate, and arterial blood pressure of rats treated with vehicle (control), T_3 (4 µg/100 g, *sc*; HyperPNT) or PTU (10 mg/100 g, *sc*; HypoPTN) for the first 30 days of life, and sacrificed at 90 days of life.

Parameters	Control	HyperPNT	HypoPNT
BW (g)	267 ± 8	211 ± 7[#]	230 ± 14[*]
VW (mg)	710 ± 20	530 ± 20[+]	659 ± 30
VW/BW (mg/g)	2.6 ± 0.1	2.5 ± 0.1	2.8 ± 0.1
T_3 (ng/mL)	0.29 ± 0.03	0.25 ± 0.05	0.30 ± 0.05
HR (bpm)	410 ± 5	393 ± 2[*]	404 ± 2
ABP (mmHg)	126 ± 2	128 ± 1	133 ± 3

Data are reported as means ± SE of 3 independent experiments for 6-8 animals/group. BW: body weight; VW: ventricular weight; HR: heart rate; ABP: arterial blood pressure; T_3: triiodothyronine; PTU: propylthiouracil. *P<0.05, #P<0.01, +P<0.001 *vs* control group (ANOVA).

indirectly activated by T_3. Moreover, the effects of TH on Mb gene expression in early postnatal life are distinct from that in adult life, when hyperthyroidism increases Mb mRNA and protein levels in heart ventricle, soleus and EDL muscles (8,9).

In neonate rats, the PTU-treatment for a 5-day period markedly decreased the ventricular Mb mRNA content, however, when the treatment was extended to 15 days, Mb mRNA was not significantly different from control values, indicating that adjustments had taken place to protect the heart against the hypothyroid status. Actually, T_3 is essential for several cyclical biochemical reactions and pathways needed for proper postnatal cardiac development. Supporting our assumption, rats treated for 15 days with PTU and reverse T_3, a competitive inhibitor of deiodinase II (D2) activity, exhibited a remarkable decrease in ventricular Mb mRNA content (data not shown). This finding demonstrates the critical role of D2 activity for the maintenance of the Mb mRNA expression in the heart. Thus, even decreased, the T_4 secreted by PTU-treated rats could provide enough T_3 to protect the heart from hypothyroidism, where D2 activity is highly increased (33). In fact, the presence of D2 has been demonstrated in human and rodent hearts (34).

Even more intriguing was the increase in skeletal muscle Mb mRNA levels in response to a 5-day PTU treatment with 5 mg/100 g BW. This may result from increased muscle contraction activity (shivering), triggered by a hypothyroidism-induced decrease in body temperature, which is known to enhance Mb gene expression. However, it is not clear if this thermoregulatory mechanism is involved in the control of neonate body temperature, in which generation of T_3 in response to increased D2 activity in brown adipose tissue is thought to be the main mechanism involved in thermogenesis (35). Nevertheless, in addition to the increased D2 activity observed in hypothyroid states and the high D2 expression in skeletal muscle, shivering could contribute to this finding. In contrast, a 2-fold increase in PTU dose led to severe hypothyroidism in the animals, with considerable loss of body weight and a hypokinetic state. Under these conditions T_4 synthesis was almost completely blocked, preventing a substantial amount of T_3 generation from T_4 deiodination in brown adipose tissue, skeletal and cardiac muscles.

In effect, Mb gene control by TH is very complex, as shown by Brik and Shainberg in 1990 (27), who demonstrated decreased Mb gene expression in isolated cardiomyocytes by T_3 treatment. In the present study, T_3 treatment from days P0 to P5 did not induce any alteration in ventricular Mb mRNA. This response was abolished when the treatment was extended to 15 days, after which the effects of T_3 were similar to those observed in both ventricular and skeletal muscles of adult rats.

On the other hand, hyperthyroidism led to an increased expression of Mb mRNA in cardiac muscle, and both hyper- and hypothyroidism caused its decrease in EDL muscle in adulthood. These results corroborate previous studies that identified THs as important regulators of many gene programs (36), and similar to the GH gene program, as pointed out in our studies. The induction of hyperthyroidism at an early postnatal period led to a decreased GH expression in adulthood, with repercussions in body weight, lean fat and bone mass (37). Taking these considerations into account, we postulate that alterations in thyroid function during the early postnatal period lead to adjustments that allow the cardiac and skeletal muscles to develop properly, even in the presence of a challenge in the intracellular environment, such as those described in models in which the nutritional environment is modified.

The hyperthyroid state increases the tissue O_2 demand, leading the heart to express more Mb, as reported. Therefore, if hyperthyroidism is induced in early postnatal life, it could define a new pattern of gene expression in the heart, which is still immature, followed by an increase in ventricular Mb mRNA expression in adulthood, a period in which a decrease in the VW and in the HR was also detected. SERCA 2 mRNA expression (unpublished data) was also increased in these animals. Taken together, these results suggest that HyperPNT improved the oxidative potential of the adult heart and its ability to remove reactive oxygen species, leading to a gain in the cardiac function, considering that the heart became smaller and bradycardic. Taking these considerations into account we postulate that rat hearts subjected to HyperPNT seem to be more prepared to act in response to a higher cardiovascular demand.

In the EDL muscle, both HyperPNT and HypoPNT led to a decrease in Mb mRNA content. EDL is a highly glycolytic muscle, therefore, such alterations seem to cause minor repercussions in its function. In contrast, the oxidative soleus muscle was preserved from the TH reprogramming effect on the Mb gene, and this could be particularly important considering its postural role.

In conclusion, the results presented here showed that Mb gene expression and the responsiveness to TH during postnatal development are tissue- and age-dependent, and indicated TH as a potential early programming agent of Mb mRNA expression in heart ventricle and skeletal muscles. The permanent decrease observed in the heart rate also reveals that HyperPNT led to repercussions in cardiovascular function, which might improve its responsiveness to increased cardiovascular demand.

Acknowledgments

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