Ventricular Myosin from Young and Adult Animals with Respect to the Thyroid State

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Abstract. Studies were conducted to analyze the effect of the thyroid hormone on ventricular myosin during ontogenesis of mice, rats and rabbits. Hypothyroidism was induced in mice and rats by administering propylthiouracyl in drinking water. Rabbits were made hyperthyroid by chronic administration of thyroxine. The change in the thyroid state of rats and rabbits influenced young and adult animals differently depending on whether V_1 or V_3 was the major ventricular isomyosin form present. Measurements of Ca^{2+}-ATPase activity of myosins from young and old control animals and from animals with changed thyroid state showed that hypothyroidism in rats is associated with a greater decrease of myosin ATPase in young rats which contain V_1 isomyosin only, when compared with old rats which contain a preponderance of V_3 isomyosin and less of the V_1 form. In rabbits, ATPase activity of ventricular myosin was more elevated after thyroxine administration in adult rabbits, which contain V_3 isomyosin only, than in young rabbits in which myosin consists of V_1 and V_3 isomyosins. Ventricular myosins of young and adult mice did not differ in their ATPase activity and the treatment of mice with propylthiouracyl had only slight effect on myosin ATPase. It can be concluded based on these results that the hypothesis concerning hypothyroidism inducing transformation of V_1 into V_3 isomyosin does not hold generally.

Key words: Ventricular myosin — Animal age — Thyroid state — ATPase activity

Introduction

The properties of ventricular myosin are under strong influence of the thyroid hormone. Hypothyroid states favor the expression of V_3 isomyosin, while thyroid hormone is required for the synthesis of the V_1 form (see Dillmann 1984;

Rupp et al. 1983). V_1 and V_3 isomyosins exhibit two- to five-fold differences in their rates of ATP hydrolysis: both the Ca^{2+}- and the actin-activated ATPase activities of V_3 are lower than those of V_1 (Hoh et al. 1978; Litten et al. 1982; Lompre et al. 1979; Lompre et al. 1981; Malhotra et al. 1981; Martin et al. 1982; Pope et al. 1980). Since ventricular myosins of different animals contain different proportions of V_1 and V_3 isomyosins, it is obvious that during hypo- or hyperthyroid states the proportions of the two isomyosins will not be altered in the same way.

The thyroid state of an animal influences the expression of atrial myosin only slightly when compared with changes observed in the ventricles (Banerjee 1983; Chizzonite et al. 1984; Dechesne et al. 1985; Samuel et al. 1986; Syrový 1986). Thus the thyroid state of an animal seems to influence atrial myosin less than does cardiac pressure overload-induced hypertrophy, which results in marked changes in the structure of atrial myosin heavy and light chains (Cummins 1982; Gorza et al. 1984; Tuchsschmid et al. 1983). Also, it has been shown (Izumo et al. 1986) with complementary DNA probes specific for six different MHC genes that the expression of the myosin heavy chain gene family in different skeletal muscle fibres of adult rats subjected to hypo- or hyperthyroidism is regulated in a highly different mode, even in the opposite direction.

It is thus obvious that the muscle response to hypo- or hyperthyroidism will be significantly different in different muscles. We tried to determine whether animal age is also a factor influencing the response of ventricular myosin to elevated or depressed levels of the thyroid hormone.

Materials and Methods

Female Wistar rats, aged 3, 44 and 74 weeks at the beginning of the experiments, white female SPF-ICR mice, aged 4 and 13 weeks at the beginning of the experiments, and domestic rabbits, aged 3 weeks and adult animals (3,500—4,000 g) at the beginning of the experiment were used.

Mice and rats were given 0.1% propylthiouracyl (PTU) in drinking water for indicated periods. Rabbits received single daily injections of L-thyroxin (0.2 mg/kg b.w.) for 14 days. The temperature was kept at 23° C.

Myosin was prepared from the ventricular part of the myocardium, the right or the left ventricles (without the septum) by the technique described by Perry (1955).

ATPase activity was measured in a Ca^{2+} medium (0.05 mol/l Tris, 5 mmol/l ATP, 10 mmol/l $CaCl_2$, 0.05 mol/l KCl, pH 7.4) at 27° C. ATPase activities were determined by measuring inorganic phosphate liberated according to the method of Fiske and SubbaRow (1925). Protein was determined by the micro Kjeldahl method.

Results

PTU was administered to young and adult rats over three different intervals to see how rats of different age respond to chemical hypothyroidism. Table 1 shows

Table 1. Body weight, heart weight, and heart-to-body weight ratio for control and PTU-treated rats. Mean ± S.D.

Preparation	Body wt (g)	Heart wt (g)	$\frac{\text{Heart wt}}{\text{Body wt}} 10^3$	Duration of PTU treatment
Control rats 31-day-old ($n = 10$)	67 ± 5.0	0.350 ± 0.03	5.2 ± 0.5	
PTU rats 31-day-old ($n = 10$)	46 ± 4.9	0.225 ± 0.02*	4.9 ± 0.39	10 days
Control rats 75-week-old ($n = 10$)	750 ± 60	1.72 ± 0.17	2.30 ± 0.20	
PTU rats 75-week-old ($n = 10$)	680 ± 59	1.556 ± 0.14	2.28 ± 0.19	10 days
Control rats 36-day-old ($n = 10$)	122 ± 12	0.460 ± 0.04	3.76 ± 0.25	
PTU rats 36-day-old ($n = 10$)	92 ± 8	0.327 ± 0.03*	3.55 ± 0.21	15 days
Control rats 76-week-old ($n = 10$)	620 ± 49	1.620 ± 0.15	2.62 ± 0.19	
PTU rats 76-week-old ($n = 10$)	610 ± 40	1.350 ± 0.12	2.22 ± 0.19	15 days
Control rats 41-day-old ($n = 10$)	180 ± 18	0.574 ± 0.004	3.18 ± 0.29	
PTU rats 41-day-old ($n = 10$)	116 ± 10	0.337 ± 0.003*	2.90 ± 0.20	21 days
Control rats 77-week-old ($n = 10$)	705 ± 51	1.80 ± 0.20	2.56 ± 0.23	
PTU rats 77-week-old ($n = 10$)	721 ± 50	1.520 ± 0.18	2.12 ± 0.20	21 days

* $P < 0.05$ (compared to control animals)

that the heart weight and the heart weight/body weight ratio are reduced in all PTU treated groups of rats. However, significant differences in heart weight were only observed between young rats treated with PTU and corresponding

control rats. The reduction of heart weight was by 38%; 29%; and 42% in young rats treated with PTU for 10; 15; and 21 days respectively.

Fig. 1 illustrates changes in Ca^{2+}-activated myosin ATPase from ventricles in young and adult rats treated with PTU. Myosin ATPase activity of normal young rats is higher than that of adult rats; this is due to the fact that ventricles of 3 to 6-week-old rats contain predominantly V_1 isomyosin with high ATPase activity, while rats around 18 months of age contain a preponderance of V_3 isomyosin with low ATPase activity and less V_1 isomyosin (Chesky and Rockstein 1977; Hoh et al. 1978; Lompre et al. 1981; Winegrad et al. 1983). PTU treatment of both young and adult rats results in a decrease of myosin ATPase, with the young rats being more affected. In young rats, ATPase activity was 57% of that measured in control animals after PTU administration for 21 days, whereas in adult rats, ATPase activity of PTU treated rats (21 days) was 74% of that in control animals. In addition, adult rats treated with PTU for 15 or 21 days showed similar reduction of myosin ATPase activities.

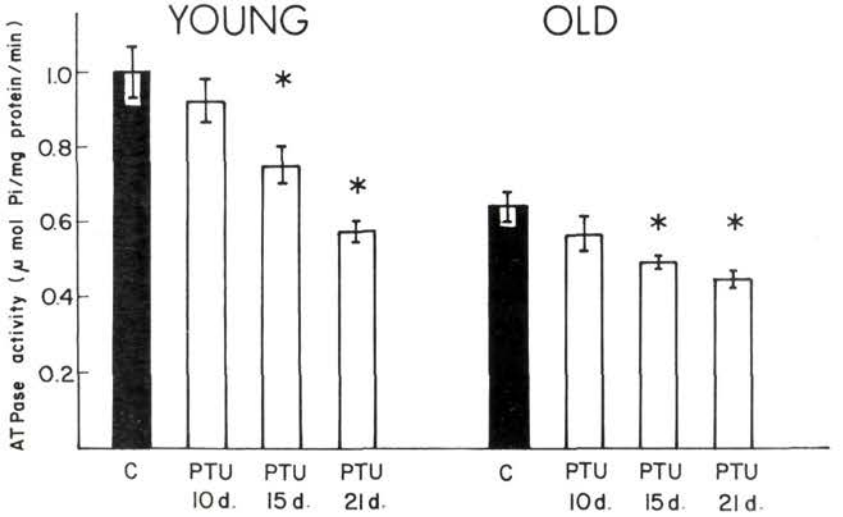

Fig. 1. Ca^{2+}-activated ATPase activity of ventricular myosin in different groups of normal and PTU-treated rats (mean ± S.D.). C-control rats, PTU-rats treated for 10, 15 and 21 days. Myosin was isolated from the same young and adult rats, used for the weight measurements (Table 1). Asterisks indicate significant differences from control rats ($P < 0.005$, $n = 3$).

Another experimental series was performed aimed at establishing whether the right and left ventricle would be influenced by PTU treatment in parallel. Rats were given PTU in drinking water for three weeks and subsequently

myosin Ca^{2+}-ATPase was determined. The results are shown in Table 2. No difference was found in myosin Ca^{2+}-ATPase activity between the left and the right ventricles of both young and adult rats; in addition, the PTU-induced depression of myosin ATPase was similar in both heart chambers.

Table 2. The effect of PTU treatment on Ca^{2+}-ATPase activity of myosin in left and right rat ventricles.

Preparation	Ca^{2+}-ATPase activity (μmol P_i/mg protein/min)
6-week-old controls, 3-week-old rats treated with PTU for 3 weeks ($n = 3$)	
R-ventricle, control	1.00 ± 0.09
L-ventricle, control	0.95 ± 0.08
R-ventricle, PTU	0.57 ± 0.04
L-ventricle, PTU	0.52 ± 0.04
10-month-old controls, 10-month-old rats treated with PTU for 3 weeks ($n = 3$)	
R-ventricle, control	0.80 ± 0.07
L-ventricle, control	0.82 ± 0.08
R-ventricle, PTU	0.63 ± 0.06
L-ventricle, PTU	0.60 ± 0.05

Experiments on rats were designed to study the effect of hypothyroid state on ventricular myocardium. To investigate hyperthyroid state effects during ontogenesis we used young and adult rabbits treated with the thyroid hormone. Table 3 shows the effect of thyroxine treatment on ventricular myosin ATPase activity. Myosin ATPase of ventricular myocardium shows higher activity in young rabbits than in adult ones. In both young and adult hyperthyroid rabbits, Ca^{2+}-ATPase of ventricular myosin was enhanced, reaching almost the same level. However, a stronger increase was seen in adult rabbits after thyroxine administration.

Table 3. Ca^{2+}-ATPase activity of ventricular rabbit myosin. The activity is expressed in terms of μmol P_i/mg protein/min. Each value represents the mean of three experiments $\pm$ S.D.

Source of myosin	Ca^{2+}-ATPase activity
5-week-old rabbit, control	0.89 ± 0.08
5-week-old rabbit, thyrotoxic	1.34 ± 0.11
Adult rabbit, control	0.51 ± 0.04
Adult rabbit, thyrotoxic	1.25 ± 0.11

In both rats and rabbits the V_1 to V_3 isomyosin ratio changes during ontogenesis. We tried therefore to establish how the change in the animal thyroid state would influence myocardium, in which no change in isomyosin distribution occurs postnatally. For these investigations young and adult mice were chosen. Mice were made hypothyroid by adding PTU into their drinking water. Table 4 shows the effect of PTU treatment on ATPase activity of mice ventricular myocardium. Ca^{2+}-ATPase activity of mice ventricular myosin was only slightly decreased after PTU administation. The body weight, the heart weight and the heart-to-body weight ratio were all effectively the same in young control and PTU treated mice; the same is true for the group of old mice (Table 5).

Table 4. Effect of PTU on myosin from ventricles of mouse heart. Activity is given in terms of μmol P_i/mg protein/min.

Source of myosin	Ca^{2+}-ATPase activity	Duration of PTU treatment
Young mice, control	1.15	
Young mice, PTU	1.12	3 weeks
Young mice, PTU	0.94	3.5 weeks
Young mice, PTU	0.85	4 weeks
Adult mice, control	1.21	
Adult mice, PTU	1.04	3 weeks
Adult mice, PTU	0.95	3.5 weeks
Adult mice, PTU	0.86	4 weeks

Table 5. Body weight, heart weight, and heart-to-body weight ratio for control and PTU-treated mice preparations

Preparation	Body wt (g)	Heart wt (g)	$\frac{\text{Heart wt}}{\text{Body wt}}(10^{-3})$
Control			
young ($n = 10$)	37.3 ± 3.1	0.167 ± 0.022	4.59 ± 0.50
old ($n = 10$)	48.4 ± 4.5	0.221 ± 0.020	4.83 ± 0.45
PTU applied for 4 weeks			
young ($n = 10$)	30.3 ± 3.9	0.146 ± 0.061	4.46 ± 0.48
old ($n = 10$)	46.0 ± 5.0	0.195 ± 0.020	4.25 ± 0.41

Discussion

The aim of this investigation was to show whether animal age is also a factor which may influence the effect of the thyroid hormone on ventricular myosin ATPase.

It is generally assumed that hyperthyroid state increases the amount of V_1 isomyosin and that hypothyroid state raises the proportion of V_3 isomyosin. In accordance with the above it might be expected that ventricular myocardium predominantly composed of V_1 and less V_3 would be more influenced by hypothyroid state than ventricular myocardium composed of more V_3 and less V_1, or ventricular myocardium containing the V_3 isoform only. This assumption was confirmed in our experiments in hypothyroid rats and hyperthyroid rabbits, but not in hypothyroid mice. In particular the treatment of rats with PTU, which results in hypothyroidism and transformation of V_1 to V_3 isomyosin (Hoh et al. 1978), reduced myosin ATPase activity in both young and adult rats; however, in young rats, which at the age of 3 to 5 weeks contain V_1 isomyosin only, the decrease of myosin ATPase was greater than in adult rats, which contain high amounts of V_3 isomyosin and a smaller proportion of V_1 isomyosin. Also, the effect of PTU administration on myosin ATPase was the same in both the left and the right ventricle, i.e. the decrease of myosin ATPase was the same in the left and right rat heart ventricle.

In rabbits, chronic administration of thyroxine, resulting in hyperthyroidism and in an increase in V_1 isomyosin, was followed by higher ATPase activity of the ventricular myosin. This elevation was higher in adult rabbits when compared with the young animals. Ventricles of adult rabbits were more affected; this is apparently due to the fact that in the hearts of 30-day-old rabbits V_1 isozyme relatively predominates over the V_3 isozyme and that V_1 is a minor component in animals older than 3 months (Litten et al. 1982).

Results obtained with venticular myosin ATPase of young and adult mice, and the effect of PTU administration on myosin ATPase of mice ventricular myosin do not confirm the general assumption that ventricular myocardium with a high proportion of V_1 isomyosin is easily influenced by PTU, resulting in a decrease of both myosin ATPase and the amount of V_1 isozyme. Our results with the same ventricular myosin ATPase of young and adult mice are in agreement with the reports by Lompre et al. (1981) showing that, starting from the age of 1 week up to 12 weeks and more, the mouse myocardium contains predominantly ventricular V_1 isomyosin throughout the life. In our experiments in both young and adult mice PTU treatment resulted in only a slight decrease of ventricular myosin ATPase activity. We have no explanation for this. The same treatment of rats resulted in a 43% reduction of myosin ATPase activity (young rats given PTU for 3 weeks, Fig. 1). We also checked whether mice and rats obtained comparable amounts of PTU in drinking water. PTU intake in drinking water did not differ substantially between mice and rats (5-week-old mice: 15.5 mg; 3-month-old mice: 16.4 mg; and 5-week-old rats: 18.7 mg PTU/ /100 g b.wt./24 h respectively.

The animal age is a factor decisive for the response of the animal to hypo-

or hyperthyroid state: young and adult animals behave differently since their ventricular myocardium differs in the isomyosin ratio. The results of our study also show that the expression of V_1 isomyosin, modulated by the thyroid hormone, and the deinducing of V_1 isomyosin by the hypothyroid state, is species specific, occuring in rats and rabbits, but not in mice. The effect of PTU on ATPase of mice ventricular myosin was not significant, although there was a tendency for myosin ATPase to decrease with prolonged PTU administration.

It is clear from the results of Chizzonite and Zak (1984), and Samuel et al. (1986) that endogenous levels of the thyroid hormone are involved in the shift from V_3 to V_1 myosin heavy chains around birth in the rat, mouse and rabbit, but not in the dog and bovine, i.e. that this shift is species specific. It has also been shown (Lompre et al. 1981) that in the rat, rabbit and pig, but not in the mouse, a change in the proportion of V_1 and V_3 isomyosin occurs postnatally. The reason why different regulatory mechanisms are found in various species is not known. There is a good correlation between the relative amount of V_1 isomyosin and the maximal shortening velocity of cardiac muscle (Schwartz et al. 1981). From the fact that ATPase activity of myosin in mice is the same in young and adult animals and that V_1 isomyosin is the only form present in mice ventricles postnatally it could be postulated that no adaptive change of myosin occurs postnatally in this species. However, it is unlikely that postnatal changes in cardiac performance occur in other mammals but not in the mouse. It is possible that in the mouse the regulation of the contractile process is not due to myosin changes, but to changes in regulatory proteins or to some other mechanism. Also the fact that PTU treatment does not change significantly ATPase activity of ventricular myosin in mice does not necessarily mean that changes in the thyroid state in mice do not concern the contractile properties of the myocardium.

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