Changes in Myosin Isoenzymes, ATPase Activity, and Contraction Duration in Rat Cardiac Muscle With Aging Can Be Modulated by Thyroxine

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To determine whether the relative decline in cardiac myosin isoenzyme V, with maturation continues progressively into senescence and whether thyroxine could reverse age-associated changes in the myosin isoenzyme profile and contraction, rats 2, 8, and 24 months old were treated with thyroxine, 6.4 mg/kg, for 7 days. Myosin isoenzymes, Ca\(^{2+}\)-myosin ATPase activities, and isometric contractile function were measured in cardiac preparations from thyroxine-treated animals and age-matched controls. Right ventricular hypertrophy did not occur with aging in controls. Thyroxine increased right ventricular weight in each age group compared to the control group. Body weight decreased by 10% in all thyroxine-treated rats. The relative right ventricular V, isoenzyme content progressively decreased from 75 ± 1% to 54 ± 1% and 14 ± 1% in controls at 2, 8, and 24 months, respectively, and was associated with a reciprocal increase in V, myosin isoenzyme. Ca\(^{2+}\)-myosin ATPase activity also progressively declined monotonically with age in the control rats from 854 ± 28 nmol Pi/mg prot/min at 2 months to 529 ± 28 nmol Pi/mg prot/min at 24 months. Thyroxine administration increased right ventricular V, at each age to 97 ± 2%, 73 ± 2%, and 59 ± 2% at 2, 8, and 24 months, respectively. A thyroxine induced increase in the Ca\(^{2+}\)-myosin ATPase activity could be detected only in the 24-month-old animals. Isometric contraction duration in thin right ventricular papillary muscles increased with age from 172 ± 4 to 180 ± 5 to 225 ± 8 ms at 2, 8, and 24 months, respectively, and was markedly shortened (p < 0.001) by thyroxine at each age so that the 24-month thyroxine value was less than the 2-month control. The maximum rate of force production did not change with age in controls and was increased by thyroxine at all ages. Thus, with aging from maturation to senescence, even in the absence of right ventricular hypertrophy, there is a profound monotonic decrease in percent right ventricular V, that is paralleled by a decline in Ca\(^{2+}\)-myosin ATPase activity. Also, neither the change in genetic expression of myosin protein synthesis nor prolonged contraction duration that occurs with aging is irreversible. Both can be modulated by thyroxine. (Circulation Research 1987;60:238-245)

The biochemistry and function of myocardial tissue has been shown to be markedly dependent on thyroid status. The hyperthyroid myocardium is characterized by a longer repolarization time of the transmembrane action potential, an increased sarcoplasmic reticulum (SR) Ca\(^{2+}\) pumping rate, an increased shortening velocity, a decrease in the isometric time to peak tension and relaxation time, elevated actin and Ca\(^{2+}\)-myosin ATPase activities, and a high percentage of myosin isoenzyme V, the "fast" form of the enzyme. Conversely, hypothyroid myocardium is characterized by prolonged contraction time, a decreased shortening velocity, a reduced SR Ca\(^{2+}\) pumping rate, reduced myosin ATPase activity, and a reduced percent V, myosin isoenzyme. The myocardium of the relatively young, i.e., maturation (approximately 200 g), euthyroid rat has a high percent of V, and a high level of ATPase activity. Thyroxine (T\(_4\)) treatment of the young euthyroid rat has been shown not to increase the Ca\(^{2+}\)-ATPase activity and its effect on the myosin isoenzyme distribution has not been studied. Rather, most studies that have examined the effect of exogenous T\(_4\) administration on myofilament properties have utilized relatively young hypothyroid rats or have avoided using the rat in favor of other species that already exhibit a low percent of V, and relatively low ATPase activities in the euthyroid state. As the euthyroid rat matures, the percent of V, decreases while the percent of V, increases, and this shift is accompanied by a decrease in the ATPase ac-
tivities in a variety of myofilament preparations. It is unknown to what extent the decline in the percent $V_t$ that occurs with aging can be reversed and the contraction duration becomes prolonged. Similarly, it is not known whether the prolonged contraction duration with aging can be modified by $T_4$.

These present studies determined 1) whether the decline in the percent $V_t$ that begins early in the rat continues progressively into senescence, 2) whether $T_4$ could reverse age-related alterations in isoenzyme pattern and $Ca^{2+}$-myosin ATPase activity, and 3) whether twitch contractile parameters that vary with age can be modulated by $T_4$, and if so, whether this could be correlated with the changes in the isoenzyme profile and ATPase activity that occur with aging or with induction of the hyperthyroid state.

Materials and Methods

Maturational (2-month), adult (8-month), and senescent (24-month) male Wistar rats obtained from the Gerontology Research Center colony were injected intramuscularly with thyroxine (Sigma Chemical Co., St. Louis, Mo.), 6.4 mg/kg initial body weight/day for 7 days. This protocol has previously been shown to induce a marked hyperthyroid (H) state. Similarly, it is not known whether the prolonged contraction duration with aging can be modified by $T_4$.

In studies examining isoenzyme distribution in selected areas of the heart, a small amount of myocardial tissue (1–10 mg) was used to obtain myosin by first placing the tissue in 50% glycerol, 1 mM sodium pyrophosphate buffer, 1 mM EDTA, 1% 2-mercaptoethanol, and 5 mM DTT at pH 8.8 for 4 hours at 0°C and then extracting the tissue overnight at 0°C in 200 µl of 50% glycerol, 50 mM sodium pyrophosphate buffer, 1 mM EDTA, 1% 2-mercaptoethanol, and 5 mM DTT at pH 8.8. The myosin-containing extract was then analyzed by electrophoresis for isoenzymes as described below.

Myosin isoenzyme determination. Myosin isoenzymes were separated on polyacrylamide gel using nondissociating conditions as described previously. A Pharmacia GE4 Electrophoresis unit was used in which the electrophoresis buffer (10% glycerol, 20 mM sodium pyrophosphate, 1 mM EDTA, 0.1% 2-mercaptoethanol at pH 8.8) was recirculated between the lower and upper chambers. The temperature in the lower chamber of the electrophoresis unit was maintained at 2°C. Myosin samples (2–4 µg) were loaded onto gel tubes containing 4% polyacrylamide. Electrophoresis was carried out for 18 hours at a voltage gradient of 14 V/cm. Following electrophoresis, gels were stained with Coomassie Blue R (0.04% in 3.5% perchloric acid) and destained with 25% 2-propanol and 7.0% acetic acid. The destained gels were scanned on a Kratos (Schoeffel Instrument) spectrodensitometer at 560 nm. The relative isoenzyme content was then determined by weighing the paper under the curve of the isoenzyme of interest and comparing it to the weight of the total curve of the isoenzymes. The amount of the heterodimer $V_2$ was divided equally between $V_1$ and $V_3$.

Myosin ATPase activity determination. Myosin $K^+\cdot Ca^{2+}$-ATPase assays were carried out in a final volume of 2 ml containing 50 mM imidazole, 500 mM KCl, 5 mM ATP, 10 mM CaCl$_2$, and 10 mM Na$_2$ at pH 7.0. Following a 10-minute incubation at 25°C the reaction was terminated by the addition of 0.5 ml (10% w/v) trichloroacetic acid (TCA) solution. The assay mixture was kept on ice for 10 minutes and then centrifuged at 2,000g in a clinical centrifuge. Inorganic phosphate in the supernatant in the steady state was measured according to the method of Taussky and Short.
Lowry et al using bovine serum albumin as the standard.

Contractile Studies

A right ventricular papillary muscle was placed horizontally in an isometric superfused muscle chamber as previously described. One muscle from each of 4 animals could be studied simultaneously in 4 muscle chambers in parallel. Right ventricular muscles were used because they were thin, discrete muscles that tolerate a superfused bath well and exhibit characteristic age changes in contractile function. There was no significant difference of CSA across age or with T treatment.

Following the experiment, each muscle was removed from the clamps, blotted and weighed. The cross-sectional area (CSA) was calculated assuming a cylindrical shape at 0.5-millisecond intervals on a Raytheon RDS500 computer. The following properties of the muscle were analyzed by a computer program: resting force, developed tension (DT), and the maximum rate of tension development (dT/dt). The time from the stimulus to peak tension (TPT) and the time from peak tension to half relaxation of peak tension (TR50) were measured. Contraction duration (CD) was defined as the sum of TPT + TR50.

Statistics

All results are expressed as mean ± standard error of n muscles in the control and treated groups. The statistical significance of age and thyroid status was determined by a least-squares means analysis of variance and linear regression analysis. The level of statistical significance was taken as p < 0.05.
animals to 14 ± 1% at 24 months. Because the values of $V_1$ and $V_3$ were derived by adding half of the value of $V_2$ to each, the increase in $V_3$ with age was the reciprocal of the decrease in $V_1$ (results not shown). No differences were observed in the percentage of $V_1$ among the right ventricular free wall, left ventricular free wall, right ventricular papillary muscle, or left ventricular papillary muscle within an age group (Figure 4).

With T$_4$ treatment, the percent of $V_1$ increased significantly at all ages (Figure 3A) with a simultaneous decrease in percent of $V_3$ (not shown). The resulting percent $V_1$ with T$_4$ treatment at each age reached the control level of the preceding younger age group. Thus, within the T$_4$-treated animals, while the percent $V_1$ still differs in 2 vs. 8 months and 8 vs. 24 months ($p < 0.001$), the magnitude of the difference between 8 and 24 months is substantially reduced compared to controls, and when considered across the entire age span, the monotonic decline with age was no longer statistically significant (Figure 3 legend).

The Ca$^{2+}$-myosin ATPase activity in control animals showed a progressive monotonic decrease with increasing age (Figure 3B). The magnitude of this decline (38%) from 2 to 24 months was substantially less than the shift in the $V_1$ isoenzyme measured over the same age range. T$_4$ reversed the age related decrease in ATPase activity in hearts from the 24-month animals which, in the controls, showed the largest Ca$^{2+}$-myosin ATPase activity decrement due to age (Figure 3B). However, T$_4$ did not appreciably increase Ca$^{2+}$-myosin ATPase activity in the younger age hearts and actually decreased it slightly (9%) in the 2-month hearts. Thus, the monotonic age-related decline in Ca$^{2+}$-ATPase activity in controls was not maintained after treatment with T$_4$. Rather, in the T$_4$ hearts the Ca$^{2+}$-ATPase activity remained fairly constant across age (Figure 3B).

The isometric contraction parameters of right ventricular papillary muscles are shown in Table 2. Resting tension did not significantly differ among groups. $dT/dt$ in the control animals did not change with age. DT increased slightly from 2 to 24 months although this change did not reach statistical significance. However, TPT (Figure 3C) and CD significantly increased with age in the control animals and RTW increased between 8 and 24 months. T$_4$ treatment increased $dT/dt$ at each age and the magnitude of this change was least in the 2-month group (25% vs. 50% in 8- or 24-month animals). The most impressive contractile changes
with T₄ occurred in the twitch duration parameters. TPT, RT₊, and CD all decreased to values less than that of the 2-month-old control rats, which had the shortest contraction times of the control animals (Figure 3C and Table 2). Among the T₄ groups, prolongation of the twitch duration with age was abolished except for TPT (Figure 3C), which still exhibited a small but significant increase with age.

Discussion

The present results demonstrate a profound reduction of the percent V₁ myosin isoenzyme from 75% at 2 months to 14% in the 24-month-old rat. This marked shift to the predominantly V₃ myosin form is similar to that which is present in other animals early in life and is also comparable to that in myocardium of young rats with induced hypothyroidism or diabetes. The magnitude of the decline in V₁ in senescence in the present study, however, is substantially greater than that reported to occur secondary to pressure overload of the ventricle in younger rats.

While it may be argued that the 24-month-old rat may have hypothyroidism, the difference in the plasma T₄ between 8 and 24 months is small and not significant although plasma T₄ decreased 50% between 2 and 8 months (Figure 1). Yet, a substantial difference in percent V₁, as well as in contractile properties, occurred between 8 and 24 months. Thus, the decrease in
percent V₁, with age most likely reflects a change in the genetic expression of the myosin ATPase isoenzyme, as can be seen with changes in the thyroid state. The specific stimulus for the decrease in the percent of isoenzyme V₁, with age could be altered loading of the heart that accompanies aging, although this has not yet been fully defined. The present study demonstrates that T₄ treatment in the control rat was able to increase the percent V₁ at all ages studied, including the 2-month-old rat in which the response of isoenzymes to T₄ treatment has not been previously reported in studies not concerned with aging. This result suggests the changes in genetic expression that occur with aging, whatever their cause, are not fixed and may be reversed to some extent under certain circumstances.

In the present study, there was no difference in myosin isoenzyme distribution between the right and left ventricle (Figure 4). There appears to be no consensus as to whether a difference exists in the isoenzyme profile between the two chambers since some studies show identical profiles in the two ventricles, while others show V₁ greater in the LV than the RV. Distribution of isoenzymes between the ventricles were also shown to vary with the species studied.

The progressive decline in myosin Ca²⁺-ATPase activity from 854 ng/mg prot/min at 2 months to 529 ng/mg prot/min in the 24-month rat paralleled the decline in percent V₁ (Figure 5A). Although an age-related decrease in actomyosin ATPase has been noted previously, the extent to which myosin Ca²⁺-ATPase activity declined in senescence has not been previously determined. The present results show that the magnitude of this depression in the 24-month-old control rat, like that of percent V₁, myosin isoenzyme, is comparable to that in hypothyroid and diabetic rats of a younger age. In the 24-month-old rat, T₄ increased the Ca²⁺-ATPase activity to the level present in the young control rat. In 2- and 8-month-old rats, T₄, in contrast to its effect on the myosin isoenzyme shift to V₁, did not increase Ca²⁺-ATPase. That Ca²⁺-ATPase activity could not be increased by thyroxine treatment in young rats has previously been noted. Failure of T₄ to stimulate myosin ATPase in the 24-month-old rat is probably because the activity prior to treatment is near maximal. The apparent discrepancy between myosin ATPase activity and percent V₁ in younger animals (Figure 5A) might be due to the variability in the assay of the former, particularly since the activity is near maximal in younger animals, coupled with a rather sensitive assay of the isomyosin forms.

Changes in the isometric twitch parameters with age observed in the euthyroid animals in the present study are similar to those reported previously with aging over the broad age span. Whether T₄ could alter contractile function in rats as young as 2 months or as old as 24 months was previously unknown. The present results show that T₄ treatment decreased TPT, RT, and CD at each age to values less than that of the 2-month-old control group, which had the shortest tim-
ing parameters of the control animals. T₄ also increased dT/dt in each age group, with the most impressive changes occurring in the two older age groups.

Simultaneous comparison of the anatomic, biochemical, and functional measurements of the present study permit a perspective on the relationships among these variables. Since relative right ventricular mass did not increase with age in the control state and since, as measured in right ventricular tissue, the percent Vₑ markedly decreased and TPT increased with age, the cardiac mass per se cannot be a determinant of the age-related change of either parameter with age in the control group. In the control state, the myosin Ca²⁺-ATPase activity varied inversely with TPT across the age span and paralleled the age-related decline in the percent Vₑ (Figure 5). This is in agreement with directionally similar changes observed in these properties among various previous studies, as well as differences in the velocity of shortening in the isotonic twitch with adult aging. The inverse correlation between contraction duration parameters, i.e., TPT and CD, and Ca²⁺-ATPase activity across the age span does not necessarily indicate a tight coupling between the two since the time course of myoplasmic free [Ca²⁺]₅₀, SR pumping rates, and the transmembrane action potential also vary with age. Similarly, the substantial increase in Ca²⁺-ATPase activity in the 24-month-old animals accompanying the reduction in TPT in response to T₄, may not be solely attributable to changes in myosin ATPase activity as T₄ also modulates other cell mechanisms that determine these parameters, among which is modulation of Ca²⁺ transport at the sarcoplasmic reticulum (SR) and sarcolemma.

While the present results demonstrate a correlation between the percent Vₑ and TPT across age in control and in T₄-treated muscles (Figure 6), the T₄ groups do not lie on the same line relating the two parameters as do the control groups but are shifted downward. This downward shift may indicate T₄ modulation of other aspects of cell Ca²⁺ metabolism in addition to an effect at the myofilament level. Thus, it appears that when myosin isoenzymes in a given muscle type differ due to changes such as age or thyroid status, excitation–contraction coupling mechanisms also differ.

In this regard, the cornerstone argument for the functional correlate of myosin ATPase, i.e., that the contraction duration (TPT in particular) among muscle types varies inversely and the velocity of shortening varies directly with the myosin ATPase activity, may require a caveat since recent evidence indicates that the rate of SR Ca²⁺ pumping, as well as myosin ATPase activity and isoenzyme profile, differs between fast and slow muscle types.

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Thyroxine Effect on Rat Myocardium

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Key Words: aging • Ca\textsuperscript{2+}-myosin ATPase activity • cardiac muscle contraction • hyperthyroid state • myosin isoenzymes


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Circ Res. 1987;60:238-245
doi: 10.1161/01.RES.60.2.238

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