Role of Thyroid Hormone in Regulation of Isomyosin Composition, Contractility, and Size of Heterotopically Isotransplanted Rat Heart

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The role of thyroid hormone on the heart in terms of contractility, induction of growth, and selective synthesis of cardiac isomyosins was studied. After transplanting rat hearts from inbred hypothyroid recipients of the same strain, two hearts were obtained in the same animal, both having reduced heart rate (200-250 bpm), decreased maximum rate of force, and high predominance of V1 isomyosin. The heart in situ carried a full load, while the transplant was denervated, beat isovolumically with minimum external work. After surgery, the recipient rats were put either on normal diet only (controls) or injected with a daily dose of T3 (average 200 μg/kg), which increased the heart rate to 340 bpm in 3 days ( euthyroid level) and to 450 bpm in 7 days (hyperthyroid level). In T3-treated rats, the contractility of both hearts normalized in 7 days and showed hyperthyroid pattern in 14 days, while the mass of the in situ hearts increased to normal values in 7 days (+130 mg) and hypertrophied in 14 days (+340 mg), in contrast to the transplanted heart, which underwent atrophy (-90 mg and -210 mg) similar to that of control group (-225 mg). The predominant V1 isomyosin was completely reversed to V3 in two weeks in both hearts. Thus, T3 can neither stimulate cardiac growth nor can it attenuate the rate of atrophy in the denervated “nonworking” heart in spite of its direct effect on contractility and synthesis of isomyosins, which was similar to that observed in the in situ heart. (Circulation Research 1987;60:824–830)
been responsible. In any event, the heterotopic iso-
transplanted heart may be a suitable model for testing
the direct effect of TH since this transplant represents
a long-term, in vivo Langendorf nonworking heart
preparation.

Based on the above, the following hypothesis is
advanced: If rats of an inbred strain were made severely
hypothyroid for a sufficient period of time, their
hearts should contain V3 isomyosins only. If the heart
of a hypothyroid donor is isotransplanted into the ab-
domen of a hypothyroid recipient, then in one animal
two hearts having V3 isomyosin only will be obtained.
One will be innervated and carry a full hemodynamic
load, while the other will be denervated and will per-
form minimal external work. If the hypothyroid state
of recipient animals could be reversed by injecting
thyroid hormone, the anticipated conversion of V3 to
V1 in both hearts could be followed. If the time course
of this conversion was found to be similar in both
hearts, then the most probable explanation of the ac-
tion of thyroid hormone on selective synthesis of iso-
myosins would be its direct effect on the myocardium.

Materials and Methods

Animal Model

The heterotopically isotransplanted heart, located in
the abdominal cavity of another inbred recipient of the
same species, was compared in its anatomical, me-
chanical, and biochemical properties with the in situ
heart of the recipient animal. Male Lewis inbred rats
(Microbiological Associates, Walkersville, Md., and
Charles River, Wilmington, Mass.) weighing 300–400 g were used in this study. The technique of heart trans-
plantation represents an adaptation of a procedure used
previously by other investigators in rats and described
in detail elsewhere.14,15 In such a preparation, the aorta
of the transplant is connected end to side to the ab-
dominal aorta of the recipient, and the pulmonary ar-
tery of the transplant is connected end to side to the
inferior vena cava of the recipient. The coronary arter-
ies of the transplanted hearts are perfused by retrograde
flow from the aorta of the recipient, and their coronary
flow drains into the recipient’s vena cava by the pump-
ing action of the right ventricle of the transplant. The
left ventricle of the transplanted heart beats against
substantially reduced afterload, the degree of which
depended on the competence of its aortic valve.

The transplanted heart resembles a long-term isovo-
lumically contracting “Langendorf preparation” in
vivo that beats at a rate between 220–260 bpm and is
perfused by blood of the recipient. It undergoes atro-
phy, which is completed within the first 2–3 weeks
after transplantation. The host heart supports full
hemodynamic load in the same animal, remains unaf-
fected by the presence of the transplant, maintains
normal heart rate (330–380 bpm), and may serve as
control heart throughout the experiment.15 The recipi-
ent animals were later anesthetized (40 mg/kg, Nem-
butal i.p.), bled, and their hearts were removed,
weighed, and dissected. The papillary muscles were
quickly removed and the rest of the hearts frozen in
liquid nitrogen for later analysis of the isomyosin spec-
trum. Our earlier studies14,15 have indicated that bio-
chemical and mechanical activities of sham-operated
controls, which did not receive the transplant, did not
differ from the host heart, and thus, the former group
was not used for comparison.

Muscle Mechanics

The rats were killed by cervical dislocation, decapi-
tated, and bled. The hearts were excised, placed in ice-
cold saline, then dissected, and the papillary muscles
carefully isolated from the left ventricle and septum.
Each papillary muscle was vertically mounted between
2 Plexiglas clamps and immersed in a constant-tem-
perature bath (27°C). The upper clamp was connected
to a Gould UC2 isometric force transducer (Statham
Instruments, Los Angeles, Calif.) by means of a stain-
less steel wire. The transducer was calibrated by 1 g and
5 g weights, which were deemed equivalent to 10 and
50 mN force, respectively. The papillary muscles
were field stimulated with square wave d-c impulses of
10-msec duration and a supramaximal voltage using a
Grass S88 stimulator (Grass Instruments, Quincy,
Mass.), through a pair of platinum electrode plates
parallel to the muscle at a frequency of 3/min (rested
state contraction). Krebs-Ringer solution containing
(in mM) NaCl 117, CaCl2 2.5, KCl 3.6, MgSO4 1.2,
NaHCO3 25, NaH2PO4 1.2, and glucose 5.5 was bub-
bled with a gas mixture of 95% O2–5% CO2. The pH of
the fluid was monitored and kept at approximately
7.40.

The muscles were first stabilized at a preload of 1 g
for 30 minutes; then, the preload was decreased to 0.25
g, and the muscles left for 10 minutes for further stabil-
ization. The preload was then increased stepwise by
0.25 g, and the length of the muscle, the developed
force, and its maximum rate of development were as-
sessed by a Grass Differentiator 7P 20C and were
recorded at each preload (Grass Recorder 7P). The
length-force relations were established, and the maxi-

Mysin Extraction

Crude myosin extracts from the apex of the left
ventricle and from the left ventricular papillary mus-
cles were prepared by extracting minced ventricular
tissue in Guba-Straub’s buffer (pH 6.5) containing 1
mM ethylenediaminetetraacetic acid (EDTA) for 30
minutes at 4°C. The suspension was centrifuged at
12,000g 5 minutes, and the supernatant (50–100 μl)
Electrophoresis of Native Myosin

Nondenaturing polyacrylamide gels were prepared as described by Pope et al., and myosin extracts (25–100 μl; 1–3 μg) were loaded onto the gels. Electrophoresis was conducted for 24 hours at a temperature between 1 and 3°C and a voltage gradient of 11 V/cm. Densitometric tracings of Coomassie brilliant blue R-stained gels were performed using a Gilford Spectrophotometer Model 240 (Gilford Instruments, Oberlin, Ohio) equipped with a Hewlett-Packard multitirecorder. The relative amounts of each isomyosin were calculated between samples taken from the apex and papillary muscles (data not given).

Statistical Analysis

Results are presented as means ± SD unless stated otherwise. Statistical differences between mean values were evaluated by Student’s t test or analysis of variance using Fisher LSD test for subsequent analysis where applicable.

Experiments

Effect of Transplantation Only on Composition of Isomyosin. Euthyroid male rats of Lewis inbred strains (Microbiological Associates and Charles River) were randomly divided into donors and recipients. After transplantation, the recipients were kept on a normal diet, and the beating of the abdominal transplants were monitored daily by palpation. The recipient animals with beating transplants were killed 2–8 weeks later to determine potential changes in composition of isomyosins in the transplants as compared with the recipient hearts and to relate these to the duration of transplantation and degree of atrophy of the transplanted heart.

Alteration of Thyroid State. In this series of experiments, 45 male Lewis inbred rats (Charles River) were fed a low iodine diet containing 0.15% propylthiouracil (Teklad Diet, Madison, Wis.) for 30 days. To verify the effect of the above diet, the spectrum of myocardial isomyosins and the contractile mechanics of isolated papillary muscles were determined in a small group of animals (n = 5). These were related to heart rate as determined from the ECG in anesthetized (40 mg/kg Nembutal i.p.) animals and to serum T3 and T4 values, which were determined by radioimmunoassay (Clinical Assay Kit, Sigma, St. Louis, Mo.). The remaining rats were then randomly divided into donors and recipients. The hearts of donors were transplanted into the abdominal cavity of recipients as described previously, and the recipient animals were then put on a normal diet. They were divided into a control group and an experimental group that, in addition to the normal diet, received daily subcutaneous injections of T3 (average dose 200 μg/kg/day). Control and experimental animals were killed 3, 7, and 14 days after surgery. The thyroid state was assessed in the first subgroup from the heart rate at the time of killing, and the daily dose of T3 for the rest of the subgroups was increased to obtain euthyroid status in the early days and hyperthyroidism later on. The thyroid status was verified by heart rate and levels of T3 and T4 in plasma, the animals were killed, the hearts quickly removed, the atria cut off, the left ventricular papillary muscles excised, and the rest of the heart weighed and frozen in liquid nitrogen.

Results

Effect of Transplantation

The proportion of the various isomyosins in the hearts of recipient animals and in their transplants was investigated in 2 groups of euthyroid rats (Microbiological Associates and Charles River) during the time interval between 16 and 56 days following surgery. The mean relative proportion of V3 isomyosin in the transplants increased from 3 to 34% in the first group and from 7 to 39% in the second group when compared with recipient hearts in situ (Figure 1). When the data obtained from individual hearts vs. time after surgery were plotted, it was found that this conversion of the spectrum of isomyosins was almost entirely accomplished within the first 2 weeks after transplantation and did not progress afterwards. When the increment of V3 in individual transplants was related to their degree of atrophy, no significant correlation could be found in either group (r = 0.325 and r = −0.257). The proportion of various isomyosins in the recipient hearts reflected the proportion observed in normal animals of the above strains, and the plasma levels of T3 and T4 of the recipient animals remained within normal limits. Similar observation also was reported in heterotopically isotransplanted hearts by Klein and Hong, published after the submission of our manuscript.

![Figure 1](image-url)
Alteration of Thyroid State

Adult inbred rats (Charles River) were first exposed to low iodine diet containing PTU for a period of 30 days, during which their mean plasma levels of T₁ and T₃ decreased respectively from 12 ± 2 nM and 55 ± 10 nM to 3 ± 0.5 nM and 15 ± 2 nM. The isometric contraction of isolated papillary muscles of 5 animals randomly selected from the above group showed a significant (39%) decrease in maximum rate of tension development and a significant (21%) increase in time to maximum tension as compared with euthyroid normal rats of the same strain (Table 1). The changes in these parameters were typical for the contractile state of the myocardium in severely hypothyroid rats.

Cardiac Isomyosins

At the time of transplantation surgery (Figure 3), the hearts of hypothyroid donors and recipients contained more than 95% of isomyosin V₃. In T₃-treated animals, a significant decrease in V₃ content occurred within 3 days in both recipient and transplanted hearts, and at 7 days, the concentration of V₃ decreased to less than 25% in both hearts. At 14 days, the V₃ isomyosin was almost entirely replaced with V₁ in both the transplant and recipient hearts. A reciprocal increase in concentration of V₁ complemented the trend of decrease in V₃ concentration.

Cardiac Mass

Administration of T₃ stimulated cardiac growth of the recipient heart (Figure 4) as manifested by the increase in heart weight on the 7th and 14th day after surgery. On the 14th day, the hearts of the T₃-treated recipients were significantly larger when compared with those on normal diet only. On the other hand, no such effect was observed in the transplant where the usual process of atrophy following the transplantation was neither reversed nor slowed down by the administration of T₃. Consequently, the decrease in cardiac mass of the transplants 14 days after surgery was the same irrespective of whether the recipients had received T₃ or not.

Table 1. Heart Rate and Tension of Isometric Contractions of Isolated Papillary Muscle from Hearts of Control and Hypothyroid Rats

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>Heart rate (per min.)</th>
<th>Developed tension (g/mm²)</th>
<th>Max. rate of tension (g/mm²/sec.)</th>
<th>Time to max. tension (msec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 5)</td>
<td>383 ± 15</td>
<td>344 ± 22</td>
<td>4.58 ± 0.26</td>
<td>38.6 ± 5.6</td>
<td>218 ± 10</td>
</tr>
<tr>
<td>Hypothyroid (n = 5)</td>
<td>342 ± 11</td>
<td>225 ± 25</td>
<td>3.42 ± 0.36</td>
<td>23.6 ± 5.0</td>
<td>264 ± 11</td>
</tr>
</tbody>
</table>

Values are mean ± SD of normal control and experimental rats after 30 days on hypothyroid diet. The differences are statistically significant in all categories (p < 0.01). The data obtained from papillary muscles were determined at Lmax, and the mean cross-sectional areas were 0.85 and 0.78 mm², respectively.
Changes in Contractile State

The heart rates and parameters of isometric contractions of isolated papillary muscles obtained from hearts of normal control and hypothyroid animals are displayed in Table 1. These data indicate significant decreases in heart rate, in maximum force and its rate of development, and prolongation of the duration of contraction in hypothyroid animals. After transplantation surgery and change to normal diet supplemented with T3 (Table 2), the maximum developed tension (DT) and time to maximum tension (TMT) of the papillary muscles isolated from hearts of hypothyroid rats (as in Table 1), and from recipient (R), and transplanted (T) hearts of animals on normal diet only (ND) or supplemented with T3. Data are expressed as percentages of the respective normal control animals of Table 1, which were taken as 100%. The number of animals is given in parentheses.

Discussion

Heterotopic isograft transplantation leads to a considerable increase in relative proportion of the V2 and V3 forms of ventricular isomyosins in the transplanted hearts of euthyroid rats in the above 2 inbred strains (Figure 1). The transplanted hearts were denervated and located in the abdominal cavity, and they beat isovolumically at a lower rate than the heart in situ. As they performed minimum external work, their load was considerably smaller, and their internal work was primarily manifested as development of intracavitary pressure with only occasional ejection of a small stroke volume.

All the above factors must be considered while attempting to explain the observed changes in the isomyosin spectrum, particularly in those situations already reported in the literature, under which a similar synthesis of V3 isomyosins occurred in the adult rat. First, the increase of pressure work as a fraction of total cardiac work19 and the chemical sympathectomy of the heart induced by administration of 6-OH dopamine20 are known to stimulate preferential synthesis of V3. Second, the fact that the transplant was located in a "foreign" environment of the body may cause subtle dedifferentiation of myocytes leading to a state that may resemble more closely the embryonic or fetal phase of development. Third, the fact that the transplant beat at a lower rate, most probably due to the lack of a tonic sympathetic discharge to its primary pacemaker, may play a role as well since the pattern of stimulation is known to influence the isomyosin com-

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Table 2. Tension of Isometric Contractions of Papillary Muscles from Hearts of Hypothyroid Rats, from Hearts of Rats on Normal Diet or Supplemented with T3

<table>
<thead>
<tr>
<th></th>
<th>Hypothyroid</th>
<th>T3 treated</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 5)</td>
<td>(n = 3)</td>
<td>(n = 3)</td>
</tr>
<tr>
<td>DT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>75</td>
<td>R 102</td>
<td>96</td>
</tr>
<tr>
<td>14 days</td>
<td>93</td>
<td>93</td>
<td>104</td>
</tr>
<tr>
<td>dT/dt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>98</td>
<td>107</td>
<td>92</td>
</tr>
<tr>
<td>14 days</td>
<td>111</td>
<td>117</td>
<td>93</td>
</tr>
<tr>
<td>TMT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 days</td>
<td>121</td>
<td>R 102</td>
<td>83</td>
</tr>
<tr>
<td>14 days</td>
<td>96</td>
<td>72</td>
<td>108</td>
</tr>
</tbody>
</table>

Mean values of maximum developed tension (DT), maximum rate of tension development (dT/dt), and time to maximum tension (TMT) of the papillary muscles isolated from hearts of hypothyroid rats (as in Table 1), and from recipient (R), and transplanted (T) hearts of animals on normal diet only (ND) or supplemented with T3. Data are expressed as percentages of the respective normal control animals of Table 1, which were taken as 100%. The number of animals is given in parentheses.
position of skeletal muscle. The relatively fast time course of the decrease in $V_f$ after transplantation surgery and its relative independence from the degree of final atrophy indicates that the stimulus has to be associated with the sudden changes to which the transplant was exposed in the early period after surgery.

The transplantation of the heart from a severely hypothyroid donor into a severely hypothyroid recipient produced a model in which both the heart in situ and the transplant contained an almost pure population of $V_f$ isomyosins. Transfer to normal diet complemented with administration of high dosages of $T_3$ led to a significant increase in the recipient heart rate, which reached the values observed in euthyroid anesthetized rats (ranging between 330 and 380 bpm). In seven days the heart rate of the recipients was within the range observed in hyperthyroid rats, which is usually between 450 and 500 bpm. The heart rate was used to assess the thyroid state of recipient animals, since the plasma values of $T_3$, and $T_4$ became meaningless due to administration of high dosages of $T_3$. The heart rate of the transplants increased significantly 3 days after administration of $T_3$ and reached the range usually observed early after transplantation of euthyroid transplants into euthyroid recipients, which is between 250 and 280 bpm in the first 3–5 days. At 7 days, the rate of the transplant reached the upper level of normal euthyroid hearts in situ, which was significantly higher than the heart rate ever observed in denervated euthyroid transplants. The transplant and recipient hearts of control animals that were on normal diet only were within their respective normal ranges commonly observed in euthyroid rats.

In view of the fact that the transplanted heart was denervated and did not support the hemodynamic load, it may be concluded that $T_3$ acted directly on the myocardium. Since it clearly affects both the myocytes, as seen from the changes in contractile state, and the pacemaker cells, as seen from the increase in heart rate, the effect of $T_3$ on isomyosin composition could be either direct only or modulated by the increased heart rate as well. The latter possibility is, however, less likely since the heart rate of transplants treated with $T_3$ reached the level observed in recipient hearts that were on the normal diet only (Figure 2). In these animals, which had the same heart rate as transplants, the $V_f$ levels increased at a considerably slower rate, and by the 14th day, it increased from 4 to 18% only as compared with the $T_3$-treated transplants, where it increased to 96%.

In spite of the parallel change in isomyosin spectrum in recipients and transplants after administration of $T_3$, no such parallelism could be observed in stimulated growth of the heart (Figure 4). The in situ hearts of hypothyroid rats are usually atrophic both in absolute terms as compared with the euthyroid animals of the same age or in relative terms as compared with euthyroid animals of the same body weight. After transplantation surgery, the transplanted heart undergoes additional atrophy that is completed within the first 3 weeks and remains stable afterwards. In our experiment (Figure 4), the mass of the recipient heart exposed to high $T_3$ plasma levels increased on the average from 615 mg to 957 mg, whereas that of the transplant decreased from 655 mg to 464 mg. This loss in mass is comparable to the loss in transplants of the control group (average weight 445 mg). The discrepancy between the growth stimulatory effect of $T_3$ on the in situ heart and lack of this effect on the transplant located in the same animal indicates that the effect of $T_3$ on the growth of the myocardium is not a direct one but requires an additional factor such as intact innervation or increased hemodynamic load. Similar conclusions were reached recently by Klein and Hong as well.

The results of our experiments support the concept that increased hemodynamic load is an important factor leading to increased protein synthesis that results ultimately in stimulated cardiac growth (for review see Wikman-Coffelt et al). Since no significant changes from normal were observed in plasma levels of $T_3$ and $T_4$ in euthyroid recipient animals, our results also show that the shift in myosin isoenzymes in favor of the $V_f$ component may occur independently of any changes in plasma level of thyroid hormones as already suggested by Dillmann. They also strongly support the concept of a direct role of thyroid hormone on antithetic regulation of gene expression as far as $V_f$ and $V_f$ distribution of isoenzymes is concerned.

On the other hand, the thyroid hormone does not seem to directly and solely increase the ratio between overall protein synthesis and degradation and does not induce stimulated growth of the heart.

In an in vitro "Langendorf preparation," the most potent stimulus for net protein synthesis appears to be, on a short-term basis, the perfusion pressure in the coronary vascular tree. Since the transplant could be considered in hemodynamic terms an in vivo Langendorf preparation, similar stimuli should produce comparable results. However, in the case of hypothyroidism in rats, the mean arterial pressure usually does not increase substantially. Therefore, the driving pressure through the vascular bed of the transplant may not have appreciably increased after administration of $T_3$ to the recipient animal. Since stimulated growth of the heart may be induced even after denervation or blockade of $\alpha$- and $\beta$-receptors, the main reason thyroid hormone could not attenuate the atrophy or induce growth of the transplanted heart would have to be the lack of increase in hemodynamic load.

References


24. Izumo S, Nadal-Ginard B, Mahdavi V: All members of MHC multigene family respond to thyroid hormone in a highly tissue specific manner. *Science* 1986;231:597–600


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