PRETREATMENT WITH 3,5,3'TRIIODO-L-THYRONINE (T₃)

Effects on myocyte contractile function after hypothermic cardioplegic arrest and rewarming

Circulating levels of 3,5,3'triiodo-L-thyronine are depressed after cardiopulmonary bypass and have been implicated to play a contributory role in the alterations in left ventricular function after hypothermic cardioplegic arrest and rewarming. The central hypothesis of the present study was that pretreatment of isolated myocytes with triiodothyronine will have a direct and beneficial effect on contractile performance after hypothermic cardioplegic arrest and rewarming. Contractile function in isolated pig left ventricular myocytes was examined by video microscopy after the following treatment protocols: (1) 37° C incubation in medium (normothermia) for 2 hours with triiodothyronine followed by a 2-hour normothermic incubation with no triiodothyronine, (2) 4 hours of normothermic incubation with no triiodothyronine, (3) normothermic incubation for 2 hours with triiodothyronine followed by 2 hours of hyperkalemic, hypothermic cardioplegic arrest ([K⁺]: 24 mmol/L; 4° C) and subsequent rewarming, and (4) normothermic incubation for 2 hours with no triiodothyronine followed by 2 hours of hyperkalemic, hypothermic cardioplegic arrest and rewarming. Two hours of normothermia with triiodothyronine increased myocyte contractile function by 30% compared with values in untreated control myocytes, and this increase persisted after a subsequent 2-hour incubation under normothermic conditions with no triiodothyronine. For example, myocyte velocity of shortening in triiodothyronine-pretreated myocytes was $84 \pm 4.9 \mu\text{m/sec}$ compared with $62 \pm 2.8 \mu\text{m/sec}$ in control myocytes ($p < 0.05$). Cardioplegic arrest and subsequent rewarming caused a significant reduction in myocyte velocity of shortening from normothermic values ($37 \pm 3.4 \mu\text{m/sec}$, $p < 0.05$). However, in myocytes pretreated with triiodothyronine, myocyte contractile function was significantly higher after hypothermic cardioplegic arrest and rewarming ($54 \pm 2.5 \mu\text{m/sec}$, $p < 0.05$). In a second series of experiments, β -adrenergic responsiveness was examined after pretreatment with triiodothyronine. In the presence of the β -adrenergic agonist isoproterenol (25 nmol/L), myocyte contractile function was increased by 26% in the triiodothyronine-treated myocytes compared with that in untreated control myocytes. This enhanced β -adrenergic responsiveness with triiodothyronine pretreatment persisted with subsequent exposure to hypothermic cardioplegic arrest and rewarming. In summary, triiodothyronine pretreatment caused an increase in myocyte contractile function and β -adrenergic responsiveness under normothermic conditions and after hypothermic cardioplegic arrest and rewarming. Thus the present study provides direct evidence to suggest that preemptive treatment with triiodothyronine may improve left ventricular contractile performance after hypothermic cardioplegic arrest and rewarming. (J THORAC CARDIOVASC SURG 1995;110:315-27)

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Received for publication July 20, 1994.

Accepted for publication Nov. 28, 1994.

*Dr. Walker is the first recipient of the Nina S. Braunwald
Research Fellowship Award.

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0022-5223/95 \$3.00 + 0 12/1/62748

The active form of thyroid hormone, 3,5,3'-triiodo-L-thyronine (T_3), which consists of two tyrosine residues coupled to iodine, is an essential factor in a wide variety of metabolic processes. Specifically, T_3 increases the active transport of ions, modulates endocrine activity, and plays an essential role in protein synthesis.¹ In addition to these widespread metabolic effects, T_3 has been shown to directly affect left ventricular (LV) pump function.²⁻⁹ For example, previous studies have shown that T_3 augments cardiac output by increasing heart rate and decreasing peripheral vascular resistance (afterload).⁸ Furthermore, T_3 has been shown to increase force production in isolated myocardial preparations.¹⁰ Clinical studies have reported a decreased circulating level of T_3 in patients with congestive heart failure, after myocardial infarction, and after cardiopulmonary bypass (CPB).^{3, 6, 11-16} Specifically, the circulating level of T_3 falls after hypothermic cardioplegic arrest and rewarming and is associated with decreased LV pump function.^{3, 6, 14}

These observations suggest that T_3 may play a mechanistic role with respect to changes in LV function after hypothermic cardioplegic arrest and rewarming.^{3, 6} Accordingly, several clinical and experimental studies have examined LV function with administration of T_3 after hypothermic cardioplegic arrest and rewarming.^{3, 6} These previous studies have demonstrated that T_3 administration after hypothermic cardioplegic arrest and rewarming improves LV pump function.^{3, 6} However, it remains unknown whether the beneficial effects that were observed with administration of T_3 after hypothermic cardioplegic arrest were mediated by a direct augmentation of myocyte contractile function. Furthermore, whether treatment with T_3 before hypothermic cardioplegic arrest has a direct effect on myocyte contractile function with rewarming has not been examined. Therefore, the present study was done to test the central hypothesis that preemptive treatment with T_3 will improve myocyte contractile function with exposure to hypothermic cardioplegic arrest and rewarming.

Hypothermic cardioplegic arrest and rewarming induces alterations in systemic loading conditions and systemic neurohormonal activation.¹⁷⁻¹⁹ Thus direct measurement of contractile performance in vivo after hypothermic cardioplegic arrest and rewarming can be problematic. This laboratory has successfully used isolated myocytes to examine the specific and direct effects of hypothermic cardioplegic arrest and rewarming on contractile processes.²⁰

That previous report demonstrated that hypothermic cardioplegic arrest and rewarming decreased myocyte contractile performance and β -adrenergic responsiveness.²⁰ The use of this isolated myocyte model to examine contractile processes after hypothermic cardioplegic arrest and rewarming, therefore, has distinct advantages. First, indices of myocyte contractile function are examined independent of the effects of the extracellular matrix and loading conditions. Second, neurohormonal influences are absent in this isolated myocyte system. Accordingly, the present study used this isolated myocyte model of hypothermic cardioplegic arrest and rewarming to examine the direct effects of pretreatment with T_3 on myocyte contractile processes.

Methods

Myocyte isolation. Six pigs (Yorkshire strain, 28 kg) were anesthetized with isoflurane (0.5%/1.5 L/min) and the lungs ventilated through a nonrecirculating anesthesia circuit. A sternotomy was then done and the heart quickly extirpated and placed in an oxygenated Krebs solution. The region of the LV free wall comprising the bed of the left circumflex coronary artery was dissected free, the artery cannulated, and the tissue prepared for myocyte isolation. All animals were treated and cared for in accordance with the National Institutes of Health "Guide for the Care and Use of Laboratory Animals."²¹

With the use of methods described by this laboratory previously,^{20, 22, 23} Krebs solution containing collagenase (0.5 mg/ml, type II; 146 U/mg, Worthington, Inc.) was perfused through the circumflex artery. The tissue was then minced and triturated with 400 μ mol/L $CaCl_2$ and collagenase (0.5 mg/ml). After filtration of the supernatant, the cells were allowed to settle and the myocyte pellet was resuspended in standard culture medium (medium 199; nutrient mixture F-12, 2 mmol/L Ca^{2+} , Gibco Laboratories, Grand Island, N.Y.). Myocytes were suspended in cell culture medium to yield a final cell concentration of 7000 viable myocytes per milliliter.

Contractile function measurement. Myocytes were imaged on an inverted microscope (Axiovert IM35, Zeiss Inc., Oberkochen, Germany) in a 2.5 ml tissue chamber with a thermoregulator to maintain the temperature of the medium at 37° C. Myocytes were stimulated at 1 Hz and contractions were imaged with use of a charge-coupled device (GPCD60, Panasonic, Secaucus, N.J.). Myocyte motion signals were input through an edge detector system (Crescent Electronics, Sandy, Utah), converted into a voltage signal, digitized, and input to a computer (80286; ZBV2526, Zenith Data Systems, St. Joseph, Mich.) for subsequent analysis.²³ Stimulated myocytes were allowed a 5-minute stabilization period after which contraction data for each myocyte were recorded from a minimum of 20 consecutive contractions. Parameters computed from the digitized contraction profiles included percentage shortening, peak velocity of shortening (in micrometers per second), peak velocity of length-

Fig. 1. Treatment protocol used in present study. Myocytes were initially placed in standard culture medium alone or with 80 pmol/L T_3 . After 2-hour incubation period in cell culture medium with or without supplementation of T_3 , one aliquot of cells from each group was subjected to 2 hours of hypothermic cardioplegic arrest, and a second aliquot from each group served as a control. In the first aliquot from each group, medium was carefully replaced with 2.5 ml of crystalloid cardioplegia solution ($[K^+]$: 24 mmol/L; pH: 7.4; oxygen tension: >400 mm Hg). These cells were maintained at 4° C for 2 hours. In the other aliquot from each group, medium was carefully replaced with fresh cell culture medium not containing T_3 and placed at 37° C for the 2-hour incubation time. At the end of this 2-hour period (normothermia or hypothermic cardioplegic arrest), myocytes were resuspended in cell medium at 37° C. Contractile function was examined at the time points designated with an X.

ening (in micrometers per second), total contraction duration (in milliseconds), and time to peak contraction (in milliseconds). Contractile measurements were obtained only on those myocytes that maintained a long axis orientation perpendicular to the microscope objective throughout the contraction profile.

Experimental design. The experimental design of this project was developed to address two specific objectives: (1) to define the acute and prolonged effects of T_3 administration on myocyte contractile function under normothermic conditions and (2) to determine whether the effects of T_3 on myocyte contractile performance persisted after exposure to hypothermic cardioplegic arrest and rewarming. The isolated myocyte suspensions were randomized to ensure uniform basal function characteristics. A schematic of the experimental design used to address these objectives is shown in Fig. 1.

Effects of T_3

Normothermic conditions. Aliquots of freshly isolated viable myocytes (2.5 ml) were randomly placed in standard cell culture medium, and steady-state baseline myocyte contractile parameters were immediately obtained. Myocytes were then randomly assigned to medium with or without 80 pmol/L T_3 (Sigma, St. Louis, Mo.), and contractile function was immediately measured in the T_3 -treated groups. Next, myocytes were incubated at 37° C for 2 hours in standard culture medium with or without supplementation of 80 pmol/L T_3 . After the 2-hour incubation period, steady-state myocyte contractile function was examined. The concentration of T_3 (80 pmol/L) and the incubation time (2 hours) were chosen on the basis of previous dose-response studies (10 pmol/L to 500 pmol/L) and incubation studies (0 to 6 hours). From the dose-response studies, the effective concentration for a 50%

response was computed to be 80 pmol/L T_3 . The maximum effects of this concentration of T_3 were observed after a 2-hour incubation period. Thus this concentration of T_3 and this incubation time were used in the present study.

Hypothermic cardioplegic arrest. After a 2-hour incubation period in cell culture medium with or without supplementation of T_3 , the myocyte groups were subjected to the second portion of the protocol. One aliquot of cardiocytes from each group was subjected to 2 hours of hypothermic cardioplegic arrest, and a second aliquot from each group served as a control. In the first aliquot from each group, medium was carefully removed and 2.5 ml of crystalloid cardioplegia solution ($[K^+]$: 24 mmol/L; pH: 7.4; oxygen tension: >400 mm Hg) was added. These cells were maintained at 4° C for 2 hours. In the other aliquot from each group, medium was carefully removed and replaced with fresh cell culture medium not containing T_3 and the cells were placed at 37° C for the 2-hour incubation time. Thus these myocytes served as a direct comparison to those exposed to hypothermic cardioplegic arrest. At the end of this 2-hour period (normothermia or hypothermic cardioplegic arrest), myocytes were resuspended in cell medium at 37° C, and contractile function was examined.

β -Adrenergic responsiveness: normothermia and hypothermic cardioplegic arrest and rewarming. β -Adrenergic receptor agonists are commonly administered at the termination of hypothermic cardioplegic arrest and rewarming to augment LV pump function. It has been previously demonstrated that T_3 administration is associated with alterations in β -adrenergic receptor number.²⁴⁻²⁶ Therefore, to determine whether administration of T_3 or exposure to hypothermic cardioplegic arrest and

Table I. Effects of incubation with T_3 on steady-state myocyte function

	0 hr incubation at 37° C	2 hr incubation at 37° C
Percent shortening (%)		
No T_3	5.3 ± 0.2	4.5 ± 0.2*
80 pmol/L T_3	6.9 ± 0.3†	5.9 ± 0.3*†
Shortening velocity (μm/sec)		
No T_3	80.9 ± 3.9	61.9 ± 2.8*
80 pmol/L T_3	114.3 ± 5.2†	83.8 ± 4.9*†
Lengthening velocity (μm/sec)		
No T_3	90.3 ± 5.7	63.5 ± 4.3*
80 pmol/L T_3	129.3 ± 8.3†	96.9 ± 8.5*†
Time to peak contraction (msec)		
No T_3	223.5 ± 7.6	263.1 ± 10.6*
80 pmol/L T_3	221.5 ± 6.7	248.0 ± 8.8*
Duration of contraction (msec)		
No T_3	459.5 ± 14.0	511.8 ± 20.3*
80 pmol/L T_3	444.8 ± 11.8	493.5 ± 17.4
Sample size		
No T_3	67	46
80 pmol/L T_3	54	49

All values presented as mean ± SEM.

* $p < 0.05$ versus 0 hours of incubation at 37° C.† $p < 0.05$ versus no T_3 .

rewarming, or both, had an effect on myocyte β -adrenergic responsiveness, myocytes from each treatment protocol were exposed to the β -adrenergic agonist isoproterenol (25 nmol/L). This concentration of isoproterenol (25 nmol/L) was previously determined by dose-response studies as the effective dose for maximum response for this isolated myocyte preparation.^{22, 23}

Myocyte geometry with hypothermic cardioplegic arrest and rewarming. To determine whether changes in myocyte morphology (that is, cell swelling) occurred with hypothermic cardioplegic arrest and rewarming or with addition of T_3 , myocyte dimensions were measured. At the specific time point for each treatment protocol (Fig. 1), an aliquot of cells was immediately placed in a fixative solution containing 2% paraformaldehyde and 2.5% glutaraldehyde fixative (325 mOsm, pH 7.4). This fixation protocol has been shown previously to have no effect on myocyte shape or volume.²⁷ Fixed myocytes were imaged with the use of Hoffman modulation contrast optics (20× objective, Modulation Optics Inc., Greenvale, N.Y.), and the cell profiles digitized with use of an image analysis system (IBAS, Zeiss Inc., Kontron, Germany). From the digitized profiles, myocyte maximum and minimum dimension and profile surface area were computed.

Data analysis. Indices of myocyte contractile function for the groups treated with and without T_3 were compared by two-way analysis of variance (ANOVA). Similarly, indices of myocyte contractile function for the normothermic group and the hypothermic cardioplegic arrest and rewarming group were compared by ANOVA. To determine whether T_3 influenced myocyte contractile function with hypothermic cardioplegic arrest and rewarming, multiway ANOVA with interactions was done. If the ANOVA detected significant differences, mean separation was done with Bonferroni bounds.²⁸ For the β -adrenergic response studies, myocyte contractile function at baseline

and after β -adrenergic stimulation was directly compared with use of a paired t test. To examine whether differences existed between β -adrenergic responsiveness and T_3 treatment after hypothermic cardioplegic arrest and rewarming, a Student's t test was done. Myocyte dimensions for T_3 treatment and hypothermic cardioplegic arrest and rewarming were compared by ANOVA. Mean separation was done with Tukey's procedure. The numbers of myocytes in each group that were used to determine maximum and minimum dimension and profile surface area conformed to a Gaussian distribution and allowed for routine parametric analysis.²⁸ All statistical procedures were done with use of the BMDP statistical software package (BMDP Statistical Software Inc., Los Angeles, Calif.). Results are presented as mean plus or minus the standard error of the mean (SEM). Values of $p < 0.05$ were considered to be statistically significant.

Results

Myocyte contractile function with T_3

Normothermia. A high yield (>70%) of viable (rod shaped, quiescent in culture) myocytes was isolated from each pig used in this study. Baseline steady-state measurements of isolated myocyte contractile function after the immediate addition of 80 pmol/L T_3 are summarized in Table I. With the acute addition of 80 pmol/L T_3 , indices of myocyte contractile function significantly increased compared with those of myocytes not exposed to T_3 (Table I). For example, 80 pmol/L T_3 increased myocyte percent shortening by 30% and increased velocity of shortening by 41%. Thus, consistent with

Table II. Effects of incubation with T_3 on myocyte function with hypothermic cardioplegic arrest and rewarming

	2 hr incubation at 37° C (matched control)*	2 hr incubation with HCA at 4° C†
Percent shortening (%)		
No T_3	4.3 ± 0.3	2.8 ± 0.2‡
80 pmol/L T_3	5.5 ± 0.3§	3.8 ± 0.1‡§
Shortening velocity (μm/sec)		
No T_3	50.9 ± 4.1	36.9 ± 3.4‡
80 pmol/L T_3	69.2 ± 5.8§	53.9 ± 2.5‡§
Lengthening velocity (μm/sec)		
No T_3	55.8 ± 6.6	35.9 ± 3.5‡
80 pmol/L T_3	88.5 ± 9.3§	53.6 ± 2.9‡§
Time to peak contraction (msec)		
No T_3	266.1 ± 9.8	238.9 ± 10.4
80 pmol/L T_3	267.8 ± 10.1	226.3 ± 8.4‡
Duration of contraction (msec)		
No T_3	526.1 ± 23.3	513.9 ± 27.9
80 pmol/L T_3	493.9 ± 24.7	443.9 ± 16.4
Sample size		
No T_3	34	42
80 pmol/L T_3	41	44

All values presented as mean ± SEM. HCA, Hypothermic cardioplegic arrest.

*Matched controls: 2 hours of incubation with or without T_3 then 2 hours with fresh medium (no T_3) at 37° C.

†Hypothermic cardioplegic arrest: 2 hours of incubation with or without T_3 , then 2 hours with cardioplegic solution (no T_3) at 4° C.

‡ p < 0.05 versus 2-hour matched controls.

§ p < 0.05 versus no T_3 .

findings of a previous recent report from this laboratory, acute administration of T_3 improves indices of myocyte contractile function.²⁹

After a 2-hour incubation at 37° C, myocyte contractile function showed a significant decline of 15% in percent shortening and 25% in velocity of shortening from initial values (Table I). However, contractile function in myocytes incubated with T_3 remained significantly higher than that in untreated myocytes and was equivalent to the function of untreated control myocytes at initial observation. However, no further augmentation of contractile performance occurred in the presence of T_3 during the incubation period. Thus the improvement in contractile performance with acute exposure to T_3 was maintained despite the time-dependent decline in contractile performance.

Hypothermic cardioplegic arrest and rewarming.

After the second 2-hour portion of the treatment protocol (either normothermic incubation in T_3 -free medium or hypothermic incubation with T_3 -free cardioplegia solution), myocyte contractile function was examined in fresh medium at 37° C in all groups (Table II). In myocytes with no T_3 pretreatment, hypothermic cardioplegic arrest and rewarming caused a marked decline in indices of contractile performance. For example, myocyte percent short-

ening declined by 36% and velocity of shortening by 38%. In myocytes preincubated with T_3 before hypothermic cardioplegic arrest and rewarming, myocyte percent and velocity of shortening remained significantly higher than respective values in untreated myocytes (37% and 45% higher, respectively) (Table II). Contractile function for normothermic control and T_3 -pretreated myocytes did not significantly change during the 2-hour incubation period.

β-Adrenergic responsiveness: normothermia and hypothermic cardioplegic arrest and rewarming.

After the specific treatments outlined in Fig. 1, contractile function in myocytes from this portion of the study was examined at baseline and after the addition of the β-adrenergic agonist isoproterenol (25 nmol/L), and the results are summarized in Tables III and IV. Consistent with previous reports from this laboratory, myocyte contractile function significantly improved after the administration of isoproterenol.^{22, 23} In myocytes pretreated with T_3 , β-adrenergic responsiveness was significantly improved immediately and after a 2-hour incubation period compared that in untreated control myocytes. As shown in Table IV, when myocytes were incubated for an additional 2 hours in standard culture medium after the initial 2-hour incubation

Table III. Effects of incubation with T_3 on myocyte β -adrenergic responsiveness

	0 hr incubation plus 25 nmol/L isoproterenol at 37° C	2 hr incubation plus 25 nmol/L isoproterenol at 37° C
Percent shortening (%)		
No T_3	10.4 $\pm$ 0.7	9.3 $\pm$ 1.1
80 pmol/L T_3	13.1 $\pm$ 0.7*	12.2 $\pm$ 0.8*
Shortening velocity (μ m/sec)		
No T_3	202.6 $\pm$ 21.3	173.9 $\pm$ 23.2
80 pmol/L T_3	275.8 $\pm$ 21.1*	256.6 $\pm$ 32.9*
Lengthening velocity (μ m/sec)		
No T_3	208.4 $\pm$ 23.7	171.2 $\pm$ 25.9
80 pmol/L T_3	269.4 $\pm$ 31.0	275.5 $\pm$ 36.9*
Time to peak contraction (msec)		
No T_3	173.1 $\pm$ 8.8	185.4 $\pm$ 18.1
80 pmol/L T_3	174.5 $\pm$ 7.3	190.1 $\pm$ 11.1
Duration of contraction (msec)		
No T_3	385.8 $\pm$ 17.6	376.8 $\pm$ 28.5
80 pmol/L T_3	386.3 $\pm$ 21.4	351.6 $\pm$ 17.5
Sample size		
No T_3	21	13
80 pmol/L T_3	19	16

All values presented as mean $\pm$ SEM.* $p < 0.05$ versus no T_3 .

with T_3 , the improvement in β -adrenergic responsiveness persisted. Finally, in myocytes pretreated with T_3 , β -adrenergic responsiveness was significantly greater than that in untreated myocytes after hypothermic cardioplegic arrest and rewarming. Thus T_3 treatment of myocytes caused a significant and persistent improvement in β -adrenergic responsiveness under normothermic conditions. More importantly, β -adrenergic responsiveness was significantly increased after hypothermic cardioplegic arrest and rewarming in myocytes that had been pretreated with T_3 .

Myocyte geometry with hypothermic cardioplegic arrest and rewarming. Dimensions for isolated myocytes immediately fixed in an isosmolar solution after each treatment protocol are shown in Table V. The frequency distributions of myocyte profile surface area with normothermia, with hypothermic cardioplegic arrest, and after subsequent rewarming are shown in Fig. 2. Myocyte size did not significantly change in normothermic myocytes treated with T_3 . In myocytes fixed at 4° C after 2 hours of hypothermic cardioplegic arrest, a significant reduction in profile surface area was observed in comparison with that of normothermic matched control myocytes (Table V). For example, myocyte profile surface area decreased from $3438 \pm 56 \mu\text{m}^2$ to $3095 \pm 60 \mu\text{m}^2$ after hypothermic cardioplegic arrest ($p < 0.05$). When myocytes were rewarmed to 37° C in standard culture medium subsequent to 2

hours of hypothermic cardioplegic arrest, myocyte size significantly increased from not only cardioplegic arrest values, but also from normothermic control values. After rewarming, mean myocyte profile surface area increased to $4440 \pm 60 \mu\text{m}^2$ ($p < 0.05$). Thus hypothermic cardioplegic arrest caused a significant reduction in myocyte size. Subsequent rewarming after hypothermic cardioplegic arrest caused myocyte swelling. Preincubation with T_3 had no effect on these changes in myocyte geometry under normothermic conditions or after hypothermic cardioplegic arrest with or without rewarming.

Discussion

Each year in the United States alone myocardial preservation techniques with the use of hypothermic cardioplegic arrest are used in more than 500,000 patients.³⁰ However, hypothermic cardioplegic arrest with cold crystalloid potassium cardioplegia and subsequent rewarming has been associated with abnormalities in LV pump function.^{17, 18, 20} Recent clinical and experimental studies have reported an improvement in LV pump function after T_3 administration at the termination of hypothermic cardioplegic arrest and rewarming.^{3, 6} However, two fundamental questions remained unanswered with respect to T_3 administration in the setting of hypothermic cardioplegic arrest and rewarming. First, it remained unknown whether T_3 administration had any direct effect on myocyte contractile performance

Table IV. Effects of incubation with T_3 on myocyte function with hypothermic cardioplegic arrest and rewarming on β -adrenergic responsiveness

	2 hr incubation at 37° C plus 25 nmol/L isoproterenol (matched control)*	2 hr incubation with HCA at 4° C† plus 25 nmol/L isoproterenol
Percent shortening (%)		
No T_3	7.7 ± 0.7	6.8 ± 0.5
80 pmol/L T_3	9.5 ± 0.6‡	8.9 ± 0.6‡
Shortening velocity (μm/sec)		
No T_3	114.5 ± 12.7	98.2 ± 9.5
80 pmol/L T_3	175.5 ± 16.3‡	134.5 ± 8.3‡§
Lengthening velocity (μm/sec)		
No T_3	122.3 ± 20.5	85.8 ± 10.8
80 pmol/L T_3	152.3 ± 12.1	114.9 ± 9.9‡§
Time to peak contraction (msec)		
No T_3	224.4 ± 17.2	205.3 ± 11.9
80 pmol/L T_3	181.1 ± 12.2	184.9 ± 8.6
Duration of contraction (msec)		
No T_3	437.3 ± 28.4	411.3 ± 28.7
80 pmol/L T_3	399.7 ± 31.6	390.8 ± 19.3
Sample size		
No T_3	16	19
80 pmol/L T_3	16	17

All values presented as mean ± SEM. HCA, Hypothermic cardioplegic arrest.

*Matched controls: 2 hours of incubation with or without T_3 then 2 hours with fresh medium (no T_3) at 37° C.

†Hypothermic cardioplegic arrest: 2 hours of incubation with or without T_3 , then 2 hours with cardioplegic solution (no T_3) at 4° C.

‡ $p < 0.05$ versus no T_3 .

§ $p < 0.05$ versus 2-hour matched controls.

Table V. Effects of T_3 administration and hypothermic cardioplegic arrest and rewarming on myocyte geometry

	2 hr incubation at 37° C	2 hr cardioplegic arrest at 4° C	2 hr cardioplegic arrest at 4° C rewarmed to 37° C
Profile surface area (μm ²)			
No T_3	3438.7 ± 55.9	3094.9 ± 59.8*	4439.7 ± 59.8*†
80 pmol/L T_3	3668.5 ± 55.7	3231.2 ± 62.2*	4452.8 ± 52.8*†
Maximum dimension (μm ²)			
No T_3	161.8 ± 1.6	118.8 ± 1.7*	176.5 ± 1.5*†
80 pmol/L T_3	167.9 ± 1.6	124.3 ± 1.4*	170.0 ± 1.3†‡
Minimum dimension (μm ²)			
No T_3	25.5 ± 0.3	28.6 ± 0.5*	30.2 ± 0.3*
80 pmol/L T_3	25.8 ± 0.3	28.6 ± 0.5*	30.4 ± 0.3*†
Sample size			
No T_3	410	285	449
80 pmol/L T_3	395	294	533

All values presented as mean ± SEM.

* $p < 0.05$ versus 2 hours of incubation at 37° C.

† $p < 0.05$ versus 2 hours of incubation at 4° C.

‡ $p < 0.05$ versus no T_3 .

in the setting of hypothermic cardioplegic arrest and rewarming. Second, it remained unknown whether preemptive treatment with T_3 would exert beneficial effects on myocyte contractile performance after hypothermic cardioplegic arrest and rewarming.

The present study attempted to address these issues by examining the direct effects of T_3 pretreat-

ment on myocyte contractile performance under normothermic conditions and after hypothermic cardioplegic arrest and rewarming. This study made three important observations. First, a 2-hour incubation with T_3 improved indices of isolated myocyte contractile function under normothermic conditions. More importantly, this improvement in myo-

Fig. 2. Isolated myocyte profile surface area was determined by digitizing cardiocyte profiles with subsequent computer analysis of results. To determine profile surface area, minimum of 300 to 500 myocytes was measured under each of following conditions: *top panel*, 2 hours of normothermia; *middle panel*, 2 hours of hypothermic cardioplegic arrest (HCA); *bottom panel*, after resuspension and rewarming (R). *Left panels* represent myocytes measured in absence of T_3 . *Right panels* represent myocytes measured after T_3 preincubation. These large sample sizes allowed for Gaussian distribution (black bell-shaped curve), thus providing means for parametric analysis. Two hours of hyperkalemic, hypothermic cardioplegic arrest caused significant reduction in myocyte volume in both panels. Subsequent reperfusion and rewarming resulted in significant increase in myocyte volume from hypothermic cardioplegic arrest values and from normothermic values. Preincubation with T_3 had no effect on alterations in cell size with either hypothermic cardioplegic arrest or with subsequent rewarming. Results are summarized in Table V.

cyte contractile performance persisted for 2 hours after removal of T_3 from the myocytes. Second, incubation with T_3 for 2 hours improved β -adrenergic responsiveness in isolated myocytes. This en-

hanced β -adrenergic responsiveness persisted for up to 2 hours after removal of T_3 from the myocytes. Third, myocytes exposed to T_3 for 2 hours followed by subsequent exposure for 2 hours to hypothermic

cardioplegic arrest and rewarming demonstrated a significant improvement in myocyte contractile function compared with that in untreated control myocytes. Furthermore, the enhancement in β -adrenergic responsiveness after 2 hours of T_3 incubation persisted after 2 hours of hypothermic cardioplegic arrest and rewarming. Thus this study for the first time provides direct evidence that pretreatment with T_3 improves myocyte contractile performance and β -adrenergic responsiveness after hypothermic cardioplegic arrest and rewarming.

Clinical and experimental studies have suggested an association between LV pump function and alterations in circulating levels of T_3 .^{3, 6, 11-16} Specifically, Hamilton and associates¹¹ reported a significant relationship between depressed levels of T_3 and alterations in LV function in patients with advanced congestive heart failure. In a clinical study, Wiersinga, Lie, and Touber¹² reported a similar decrease in T_3 levels for 3 days after myocardial infarction with hemodynamic compromise. In an experimental study, Buccino and associates³¹ reported that the maximum velocity of isotonic shortening in feline papillary muscles varied directly with the thyroid state. Thus previous clinical and experimental studies have provided evidence to suggest that T_3 may be an important determinant in overall LV performance.²⁻⁹ Consistent with a recent report, results from the present study demonstrated a positive effect of T_3 treatment on myocyte contractile function.²⁹ More important, pretreatment with T_3 for 2 hours caused a persistent improvement in myocyte contractile function. Thus these results suggest that short-term T_3 pretreatment may have prolonged and beneficial effects on myocyte contractile performance under normothermic conditions.

The use of hypothermic, hyperkalemic cardioplegia is the most common method of myocardial protection during cardiac operation. However, transient LV dysfunction can occur in the perioperative setting.¹⁷⁻¹⁹ Several clinical and experimental studies have demonstrated an association between decreased LV function and T_3 levels after hypothermic cardioplegic arrest and rewarming.^{3, 6, 16} In light of this association between decreased levels of T_3 and poor LV pump function after hypothermic cardioplegic arrest and rewarming, several clinical and experimental studies have examined the effects of the acute administration of T_3 on global indices of LV function after CPB.^{3, 6, 14} For example, Novitzky, Human, and Cooper³ reported that in pigs treated with T_3 , cardiac output was 30% higher than that in

untreated control animals at the termination of CPB. However, a direct causal relation between T_3 and LV function after hypothermic cardioplegic arrest and rewarming has been difficult to establish in light of the associated changes that occur in systemic loading conditions and neurohormonal systems.¹⁷⁻¹⁹ The present study, therefore, examined the direct effects of T_3 administration on myocyte contractile function after hypothermic cardioplegic arrest and rewarming. Pretreatment with T_3 for 2 hours followed by 2 hours of hypothermic cardioplegic arrest and rewarming significantly improved myocyte contractile function compared with that in myocytes with no T_3 treatment. Thus this study provides direct evidence that the improvement in LV function that was observed in previous reports with T_3 administration after CPB may be a result of a direct effect of T_3 on myocyte contractile function.

The present study examined the potential interactive effects of T_3 treatment and β -adrenergic responsiveness under normothermic conditions and after hypothermic cardioplegic arrest and rewarming. These studies have potential clinical relevance for two reasons. First, an increasing number of patients are seen for cardiac operation with advanced degrees of LV dysfunction.³⁰ Chronic LV failure has been clearly associated with alterations in the β -adrenergic receptor system and an associated reduction in β -adrenergic responsiveness.³²⁻³⁵ Second, the transient LV dysfunction after hypothermic cardioplegic arrest and rewarming is commonly treated with β -adrenergic receptor agonists.³⁶ However, hypothermic cardioplegic arrest and rewarming has been shown to cause a decrease in β -adrenergic receptor density and alterations in β -adrenergic receptor transduction.³⁷⁻³⁹ Thus therapeutic strategies that improve β -adrenergic responsiveness after hypothermic cardioplegic arrest and rewarming will have particular clinical importance. Previous reports have demonstrated that T_3 acutely modulates β -adrenergic receptor density and responsiveness.^{24, 25} For example, Chang and Kunos²⁵ demonstrated that acute administration of T_3 increased the chronotropic response of rat myocytes to β -adrenergic stimulation. Furthermore, a recent report from this laboratory demonstrated that acute administration of T_3 in a control, normothermic cardiocyte preparation improved β -adrenergic responsiveness.²⁹ The present study builds on these previous reports by demonstrating that the improvement in β -adrenergic responsiveness after T_3 administration was not only an acute effect, but also caused prolonged and

beneficial effects on myocyte β -adrenergic responsiveness after T_3 removal under normothermic conditions. More important, the present study demonstrated that myocytes pretreated with T_3 followed by hypothermic cardioplegic arrest and subsequent rewarming exhibited enhanced β -adrenergic responsiveness compared with untreated myocytes. The unique findings from this portion of the present study suggest that T_3 pretreatment may provide a novel approach in enhancing β -adrenergic responsiveness in the clinical setting of hypothermic cardioplegic arrest and rewarming.

Hypothermic, hyperkalemic cardioplegic arrest and rewarming has long been associated with myocardial edema.⁴⁰⁻⁴³ For example, Laks and colleagues⁴¹ reported an increase in extravascular water content after CPB with hemodilution and hypothermia in canine hearts. Weng and colleagues⁴⁰ reported an increase in myocardial water content in isolated porcine hearts after coronary perfusion with cardioplegic solutions. The increase in myocardial water content that occurs after hypothermic cardioplegic arrest and rewarming has been associated with abnormalities in LV compliance.⁴⁰⁻⁴³ However, it remained unclear from these previous studies whether the increased myocardial water content after hypothermic cardioplegic arrest and rewarming was intracellular, extracellular, or both.⁴⁰ Previous reports have demonstrated ultrastructural alterations within the myocyte after CPB or global ischemia with subsequent reperfusion.⁴¹ Drewnowska, Clemon, and Baumgarten⁴⁴ reported alterations in rabbit myocyte volume regulation after short exposure times to hyperkalemic, hypothermic cardioplegic arrest. That study demonstrated that the presence of magnesium, alterations in chloride transport, and inhibition of the sodium/potassium adenosinetriphosphatase would significantly influence volume regulatory processes with hyperkalemic hypothermic cardioplegic arrest and rewarming.⁴⁴ The present study demonstrated that myocyte profile surface area, which is directly proportional to myocyte volume,⁴⁵ significantly decreased after hyperkalemic, hypothermic cardioplegic arrest and significantly increased after subsequent rewarming. Thus this study provides direct evidence that myocyte swelling may be a contributing factor in the increased myocardial water content reported in previous *in vivo* studies.

Maintenance of cell volume occurs through a balance among factors that contribute to osmotic equilibrium. These include the integrity of the sar-

colemma and its permeability to various ions and membrane pumps such as the sodium/potassium and sodium/potassium/chloride pumps.⁴⁶ Alterations in the membrane, as with ischemia, lead to alterations in permeability and pump function with resultant cell swelling or shrinkage. Specifically, with cell swelling, there is an efflux of electrolytes through activation of potassium channels and anion channels, probably by an increase in intracellular calcium activity.⁴⁶ Whalen and associates⁴⁷ described alterations in sodium/potassium pump activity and increases in sarcolemmal permeability to sodium and calcium after ischemia. Hyperkalemic cardioplegic arrest maintains the myocyte in a depolarized state (more positive resting potential) and therefore prevents excitation/contraction coupling. The depolarized environment results in an influx of sodium into the myocyte with a resultant increase in intracellular calcium levels by the sodium/calcium exchanger.⁴⁸ Thus significant alterations in ionic homeostasis occur with prolonged hyperkalemic cardioplegic arrest,⁴⁰⁻⁴³ which in turn cause abnormalities in volume regulation with subsequent rewarming. In the present study, the increased myocyte volume observed after hypothermic cardioplegic arrest and rewarming was probably a result of alterations in volume regulation. In light of the fact that T_3 has been shown to acutely regulate the flux of ions, glucose, and small amino acids across the sarcolemma,^{1, 49-51} we suspected that T_3 may influence the changes in ionic homeostasis during hypothermic cardioplegic arrest and therefore modulate volume regulation with subsequent rewarming. However, pretreatment of myocytes with T_3 did not significantly affect the changes in myocyte volume after hypothermic cardioplegic arrest and rewarming. Two important conclusions can be made from this portion of the study. First, myocyte swelling that occurs after hypothermic cardioplegic arrest and rewarming indicates abnormalities in volume regulation processes, which in turn may be associated with abnormalities in contractile processes. Second, the improvement in myocyte contractile function that was observed after hypothermic cardioplegic arrest and rewarming after T_3 pretreatment was probably not a result of modulation of volume regulatory processes.

The administration of T_3 and hypothermic cardioplegic arrest and rewarming *in vivo* are associated with alterations in systemic loading conditions and neurohormonal activation.^{2, 17-19} In the present study, an *in vitro* system was used to eliminate the

influence of systemic loading conditions and neurohormonal alterations. Thus, by virtue of the experimental design, the systemic effects of T_3 pretreatment could not be addressed. Second, the present study examined the effects of T_3 pretreatment with use of a fixed dosage and time interval, which were selected on the basis of the results of preliminary studies. However, it remains unclear whether the concentration of T_3 and the duration of treatment used in the present study could be applied to an *in vivo* model. In light of the findings from the present study that demonstrated the direct and beneficial effects of T_3 pretreatment in the setting of hypothermic cardioplegic arrest and rewarming, future *in vivo* studies would be appropriate to address these issues. In a previous report, Urabe and associates⁵² examined isolated myocyte contractile function in an unloaded state and after viscous loading of the myocyte. That study demonstrated intrinsic differences in contractile performance between hypertrophied and normal cardiocytes with use of this technique.⁵² In the present study the effects of T_3 treatment on the ability of the myocyte to contract against an external load were not addressed. Finally, specific mechanisms by which pretreatment of myocytes with T_3 exerted the direct and beneficial effects observed on myocyte contractile performance under normothermic conditions and after hypothermic cardioplegic arrest and rewarming remain unclear. It is recognized that long-term administration of T_3 (>72 hours) induces specific alterations in myocyte contractile protein messenger ribonucleic acid expression and protein synthesis.⁵³⁻⁵⁵ These chronic effects of T_3 are transduced by binding to nuclear receptors with resultant alterations in transcription rates for contractile protein transcription and translation.⁵³⁻⁵⁵ It has been demonstrated that T_3 causes a significant increase in the expression of the *c-myc* protooncogene and increased mitochondrial protein synthesis.^{1, 56-58} However, it is unlikely that the mechanisms for the acute increase in myocyte contractile function with T_3 observed in the present study were mediated by alterations in contractile protein transcription and translation. Acute administration of T_3 can mediate intracellular regulatory processes within 1 hour of exposure.⁵⁶ Other acute effects of T_3 have been reported, which include (1) increased sodium channel bursting,^{51, 59} (2) increased Ca^{+2} adenosinetriphosphatase activity,^{60, 61} (3) increased adenylate cyclase activity,⁶² and (4) altered sarcolemmal permeability to small ions, glucose, and amino acids.^{1, 49-51} In the present study, T_3

failed to prevent the volume regulatory changes after hypothermic cardioplegic arrest and rewarming. Therefore the mechanism of action for the acute effects of T_3 on myocyte contractile processes is probably not caused by alterations in ion flux in the cardiocyte, but rather through alternative mechanisms. In light of the findings from the present study in which T_3 had significant and prolonged effects on myocyte contractile performance, elucidation of the basic mechanisms responsible for these effects is warranted.

REFERENCES

1. Dillman WH. Mechanisms of thyroid hormone action. *Med Clin North Am* 1985;69:849-61.
2. Morkin E, Flink IL, Goldman S. Biochemical and physiologic effects of thyroid hormone on cardiac performance. *Prog Cardiovasc Dis* 1983;25:435-61.
3. Novitzky D, Human PA, Cooper DK. Inotropic effect of triiodothyronine following myocardial ischemia and cardiopulmonary bypass: an experimental study in pigs. *Ann Thorac Surg* 1988;45:50-5.
4. Holland FW, Brown PS, Clark RE. Acute severe postischemic myocardial depression reversed by triiodothyronine. *Ann Thorac Surg* 1992;54:301-5.
5. Dyke CM, Yeh T, Lehman JD, et al. Triiodothyronine-enhanced left ventricular function after ischemic injury. *Ann Thorac Surg* 1991;52:14-9.
6. Novitzky D, Cooper DK, Barton CI, et al. Triiodothyronine as an inotropic agent after open heart surgery. *J THORAC CARDIOVASC SURG* 1989;98:972-8.
7. Wechsler AS, Kadletz M, Ding M, Abd-Elfattah A, Dyke C. Effects of triiodothyronine on stunned myocardium. *J Card Surg* 1993;8:338-41.
8. Feldman T, Borow KM, Sarne DH, Newmann A, Lang RM. Myocardial mechanics in hyperthyroidism: importance of left ventricular loading conditions, heart rate and contractile state. *J Am Coll Cardiol* 1986;7:967-74.
9. Klein I. Thyroid hormone and the cardiovascular system. *Am J Med* 1990;88:631-7.
10. Snow TR, Deal MT, Connelly TS, Yokoyama Y, Novitzky D. Acute inotropic response of rabbit papillary muscle to triiodothyronine. *Cardiology* 1992;80:112-7.
11. Hamilton MA, Stevenson LW, Luu M, Walden JA. Altered thyroid hormone metabolism in advanced heart failure. *J Am Coll Cardiol* 1990;16:91-5.
12. Wiersinga WM, Lie KI, Touber JL. Thyroid hormones in acute myocardial infarction. *Clin Endocrinol* 1981;14:367-74.
13. Hamilton MA. Prevalence and clinical implications of abnormal thyroid hormone metabolism in advanced heart failure. *Ann Thorac Surg* 1993;56:S48-53.
14. Novitzky D, Human PA, Cooper DK. Effect of tri-

- iodothyronine on myocardial high energy phosphates and lactate after ischemia and cardiopulmonary bypass. *J THORAC CARDIOVASC SURG* 1988;96:600-7.
15. Pederson F, Perrild H, Rasmussen SL, Skovsted L. Low T₃ syndrome in acute myocardial infarction: relationship to β -adrenergic blockade and clinical course. *Eur J Clin Pharmacol* 1984;26:669-73.
16. Holland FW, Brown PS, Clark RE. Acute severe postischemic myocardial depression reversed by triiodothyronine. *Ann Thorac Surg* 1992;54:301-5.
17. Roberts AJ, Spies SM, Sanders JH, et al. Serial assessment of left ventricular performance following coronary artery bypass grafting. *J THORAC CARDIOVASC SURG* 1981;81:69-84.
18. Phillips HR, Carter JE, Okada RD, et al. Serial changes in left ventricular ejection fraction in the early hours after aortocoronary bypass grafting. *Chest* 1983;83:28-34.
19. Engelman RM, Rousou JH, Lemeshow S, Dobbs WA. The metabolic consequences of blood and crystalloid cardioplegia. *Circulation* 1981;64(Suppl):II67-74.
20. Handy JR, Spinale FG, Mukherjee R, Crawford FA. Hypothermic potassium cardioplegia impairs myocyte recovery of contractility and inotropy. *J THORAC CARDIOVASC SURG* 1994;107:1050-8.
21. Committee on the care and use of laboratory animals. Guide for the care and use of laboratory animals. Washington, D.C.: National Research Council Institute of Laboratory Animal Resources, 1985; NIH publication no. 86-23.
22. Spinale FG, Zellner JL, Tomita M, Crawford FA, Zile MR. Relationship between ventricular and myocyte remodeling with the development and regression of supraventricular tachycardia induced cardiomyopathy. *Circ Res* 1991;69:1058-67.
23. Mukherjee R, Crawford FA, Hewett KW, Spinale FG. Cell and sarcomere contractile performance from the same cardiocyte using video microscopy. *J Appl Physiol* 1993;74:2023-33.
24. Williams LT, Lefkowitz RJ, Wantanabe AM, Hathaway DR, Besch HR. Thyroid hormone regulation of β -adrenergic receptor number. *J Biol Chem* 1977;252:2787-9.
25. Chang HY, Kunos G. Short term effects of triiodothyronine on rat heart adrenoceptors. *Biochem Biophys Res Commun* 1981;100:313-20.
26. Hammond HK, White FC, Buxton ILO, Saltzstein P, Brunton LL, Longhurst JC. Increased myocardial beta-receptors and adrenergic responses in hyperthyroid pigs. *Am J Physiol* 1987;252:H283-90.
27. Gerdes AM, Kriseman J, Bishop SP. Morphometric study of cardiac muscle: the problem of tissue shrinkage. *Lab Invest* 1982;46:271-4.
28. Steel RGD, Torrie JH. Principles and procedures of statistics: a biometrical approach. 2nd ed. New York: McGraw-Hill, 1980.
29. Walker JD, Crawford FA, Mukherjee R, Zile MR, Spinale FG. The direct effects of acute administration of 3,5,3'-triiodo-L-thyronine on myocyte contractile function and β -adrenergic responsiveness. *Ann Thorac Surg* 1994;58:851-6.
30. Schneidman K, Peebles R. Socio-economic fact book for surgery. Chicago: American College of Surgeons, 1991-1992:46-7.
31. Buccino RA, Spann JF, Pool PE, Sonnenblick EH, Braunwald E. Influence of the thyroid state on the intrinsic contractile properties and energy stores of the myocardium. *J Clin Invest* 1967;46:1669-82.
32. Tanaka R, Fulbright BM, Mukherjee R, et al. The cellular basis for the blunted response to β -adrenergic stimulation in supraventricular tachycardia-induced cardiomyopathy. *J Mol Cell Cardiol* 1993;25:101-19.
33. Bristow MR. Changes in myocardial and vascular receptors in heart failure. *J Am Coll Cardiol* 1993; 22(Suppl):I213-21.
34. Bristow MR, Ginsburg R, Umans V, et al. β_1 and β_2 Adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective β_1 -receptor down regulation in heart failure. *Circ Res* 1986;59:297-309.
35. Fowler MB, Laser JA, Hopkins GL, Minobe W, Bristow MR. Assessment of the β -adrenergic receptor pathways in the intact failing human heart: progressive receptor down-regulation and subsensitivity to agonist response. *Circulation* 1986;74:1290-302.
36. Steen PA, Tinker JH, Pluth JR, Barnhorst DA, Targhan S. Efficacy of dopamine, dobutamine, and epinephrine during emergence from cardiopulmonary bypass in man. *Circulation* 1978;57:378-84.
37. Schwinn DA, Leone BJ, Spahn DR, et al. Desensitization of myocardial β -adrenergic receptors during cardiopulmonary bypass: evidence for early uncoupling and late downregulation. *Circulation* 1991;84:2559-67.
38. Schranz D, Droege A, Broede A, et al. Uncoupling of human cardiac β -adrenoreceptors during cardiopulmonary bypass with cardioplegic cardiac arrest. *Circulation* 1993;87:422-6.
39. Sakagoshi N, Matsuda H, Watanabe Y, et al. Alterations in the β -adrenergic receptor system after hypothermic ischemia in hearts with preischemic β -receptor desensitization. *J Surg* 1992;53:66-73.
40. Weng Z-C, Nicolosi AC, Detwiler PW, et al. Effects of crystalloid, blood, and University of Wisconsin perfusates on weight, water content, and left ventricular compliance in an edema-prone, isolated porcine heart model. *J THORAC CARDIOVASC SURG* 1992;103:504-13.
41. Laks H, Standeven J, Blair O, Hahn J, Jellinek M, Willman VL. The effects of cardiopulmonary bypass with crystalloid and colloid hemodilution on myocardial extravascular water. *J THORAC CARDIOVASC SURG* 1977;73:129-38.

42. Utley JR, Michalsky GB, Bryant LR, Mobin-Uddin K, McKean HE. Determinants of myocardial water content during cardiopulmonary bypass. *J THORAC CARDIOVASC SURG* 1974;68:8-16.
43. Fogalia RP, Steed DL, Follette DM, DeLand E, Buckberg GD. Iatrogenic myocardial edema with potassium cardioplegia. *J THORAC CARDIOVASC SURG* 1979;78:217-22.
44. Drewnowska K, Clemon HF, Baumgarten CM. Prevention of myocardial intracellular edema induced by St. Thomas' Hospital cardioplegic solution. *J Mol Cell Cardiol* 1991;23:1215-21.
45. Houser SR, Freeman AR. A simple method for volumetric measurements in isolated cardiac muscle. *Am J Physiol* 1979;236:H519-24.
46. Lang F, Ritter M, Volkl H, Haussinger D. The biologic significance of cell volume. *Renal Physiol Biochem* 1993;16:48-65.
47. Whalen DA, Hamilton DG, Ganote CE, Jennings RB. Effect of a transient period of ischemia on myocardial cells. *Am J Pathol* 1974;74:381-98.
48. Scheu SS, Blaustein MP. Sodium/potassium exchange and control of cell calcium and contractility in cardiac and vascular smooth muscle. In: Fozard HA, Haker E, Jennings RB, Katz AM, Morgan HE, eds. *The heart and cardiovascular system*. New York: Raven Press, 1992:903-44.
49. Segal J. Action of the thyroid hormone at the level of the plasma membrane. *Endocr Res* 1989;15:619-49.
50. Gordon A, Schwartz H, Gross J. The stimulation of sugar transport in heart cells grown in a serum free medium by picomolar concentrations of thyroid hormones: the effects of insulin and hydrocortisone. *Endocrinology* 1986;118:52-7.
51. Dudley SC, Baumgarten CM. Bursting of cardiac sodium channels after acute exposure to 3,5,3'-triiodo-L-thyronine. *Circ Res* 1993;73:301-13.
52. Urabe Y, Mann DL, Kent RL, et al. Cellular and ventricular contractile dysfunction in experimental canine mitral regurgitation. *Circ Res* 1992;70:131-47.
53. Oppenheimer JH. Thyroid hormone action at the nuclear level. *Ann Intern Med* 1985;102:374-84.
54. Oppenheimer JH. Thyroid hormone action at the cellular level. *Science* 1979;203:971-9.
55. Sterling K. Thyroid hormone action at the cell level. *N Engl J Med* 1979;300:117-23.
56. Robbins RJ, Swain JL. C-myc protooncogene modulates cardiac hypertrophic growth in transgenic mice. *Am J Physiol* 1992;262:H590-7.
57. DeNayer P. Thyroid hormone action at the cellular level. *Horm Res* 1987;26:48-57.
58. Sterling K, Milch PO. Thyroid hormone binding by a component of mitochondrial membrane. *Proc Natl Acad Sci* 1975;72:3225-9.
59. Craelius W, Green WL, Harris DR. Acute effects of thyroid hormone on sodium currents in neonatal myocytes. *Biosci Rep* 1990;10:309-15.
60. Rudinger A, Mylotte KM, Davis PJ, Davis FB, Blas SD. Rabbit myocardial membrane Ca^{+2} -adenosine triphosphate activity: stimulation in vitro by thyroid hormone. *Arch Biochem Biophys* 1984;229:379-85.
61. Suko J. The calcium pump of cardiac sarcoplasmic reticulum: functional alterations at different levels of thyroid state in rabbits. *J Physiol* 1973;228:563-82.
62. Levey GS, Epstein SE. Myocardial adenyl cyclase: activation by thyroid hormones and evidence for two adenyl cyclase systems. *J Clin Invest* 1969;48:1663-9.

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