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Cardiac Effects of 3,5-Diiodothyropropionic Acid, a Thyroid Hormone Analog with Inotropic Selectivity[1]

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ABSTRACT

Thyroid hormone exerts a strong positive inotropic action on the heart and induces α-myosin heavy chain (MHC) gene expression. 3,5-Diiodothyropropionic acid (DITPA), a carboxylic acid analog with low metabolic activity, was observed to induce α-MHC mRNA in heart cell culture with $EC_{50} \approx 5 \times 10^{-7}$ M. To determine if the compound has positive inotropic actions, the effects of DITPA and L-thyroxine on heart rate, left ventricular pressures, left ventricular *dP/dt*, myosin isoenzymes and hepatic α-glycerolphosphate dehydrogenase activity were compared in hypothyroid rats. Binding affinities of DITPA and triiodothyronine for bacterially expressed *alpha*-1 and *beta*-1 thyroid hormone receptors (TRs) also were determined. Over the dosage range of 150 to 1500 μg/100 g, DITPA produced increases in left ventricular *dP/dt* comparable to those obtained with L-thyroxine at dosages of 1.5 to 15 μg/100 g, but with significantly less tachycardia. The increase in α-MHC mRNA was about the same with both compounds whereas α-MHC protein content and GPDH activity increased less with DITPA. These differences could not be explained by preferential binding of DITPA to TR subtypes. Because heart rate is a major determinant of myocardial oxygen consumption, DITPA is able to achieve increased cardiac performance at lower myocardial oxygen costs.

Thyroid hormone has been known for many years to have direct chronotropic and inotropic actions (Morkin *et al.*, 1983). In addition, thyroid hormone plays an essential role in controlling metabolic processes within the heart, including amino acid and electrolyte transport, carbohydrate, protein and lipid metabolism and regulation of the rate of oxidative phosphorylation. Whereas the mechanisms responsible for this broad range of effects remain uncertain, research over the past decade has provided evidence that many of the actions of thyroid hormone are mediated by controlling the expression of specific genes (Samuels *et al.*, 1988). These effects on gene expression are now thought to be initiated through binding to nuclear thyroid hormone receptors (TRs) that have been identified as the products of the c-*erbA* protooncogenes (Evans, 1988; Samuels *et al.*, 1988).

A well-studied example of this mechanism of thyroid hormone action is the regulation of cardiac MHC mRNAs. In ventricular myocardium of rats and rabbits, thyroid hormone causes accumulation of α-MHC mRNA and suppresses expression of β-MHC mRNA (Gustafson *et al.*, 1986; Izumo *et al.*, 1986). The result is an increase in the level of the high-ATPase activity V_1 (α,α) myosin isoform and a decrease in the low activity V_3 (β,β) form. The induction of the V_1 isoenzyme has been thought to be physiologically important because the V_1 content of the heart in various species correlates closely with the intrinsic speed of contraction (Swynghedauw, 1986). When thyroid hormone is administered exogenously, however, the onset of the inotropic response occurs rapidly, generally within 12 hr (Brooks *et al.*, 1985), preceding changes in myosin composition (Gay *et al.*, 1987). Thus, the thyroid hormone-induced increase in the V_1 myosin isoform serves as a biochemical marker associated with the positive inotropic action of the hormone, but additional mechanisms must be involved, particularly in the initial phase of the response.

Other effects of thyroid hormone, such as enhancement of amino acid and sugar transport and stimulation of mitochondrial ATP synthesis, occur in the absence of protein synthesis (Sterling *et al.*, 1980). These actions have been explained by binding of T_3, the intracellular form of the hormone, to extranuclear receptors located in mitochondria and cell membranes.

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ABBREVIATIONS: MHC, myosin heavy chain; ATPase, adenosine triphosphatase; T_3, 3,5,3′-triiodo-L-thyronine; Triac, 3,5,3′-triiodothyroacetic acid; GPDH, α-glycerolphosphate dehydrogenase; DITPA, 3,5-diiodothyropropionic acid; LV, left ventricular; T_4, L-thyroxine; LVSP, LV systolic pressure; SR, sarcoplasmic reticulum.

Specific binding proteins have been identified in plasma membranes (Rudinger *et al.*, 1984; Mylotte *et al.*, 1985;), endoplasmic reticulum (Cheng *et al.*, 1987) and cytosol (Kato *et al.*, 1989). Interestingly, the relative potency of thyroid hormone analogs for various extranuclear receptors is reported not to correlate well with their affinity for binding to nuclear receptors (Davis *et al.*, 1983). The significance of these extranuclear receptor sites has remained controversial, however, because functional activity frequently has been demonstrated only at concentrations of thyroid hormone higher than those found in the euthyroid state.

Despite the diversity of thyroid hormone actions and the apparent multiplicity of its binding sites, there has been little success in identifying thyroid hormone analogs with selective actions. A thyroid analog with selective positive inotropic actions might be useful in management of congestive failure, particularly if relatively free of undue stimulation of myocardial oxygen consumption. In an earlier study, the carboxylic acid analog of T_3, Triac, was found to have the most favorable ratio of V_1 induction to stimulation of GPDH activity among the analogs tested (Sheer and Morkin, 1984). Recently, we observed that the carboxylic acid analog, DITPA, which has been reported to be metabolically inactive as assessed by its effects on oxygen consumption and suppression of goiter formation (Stasilli *et al.*, 1959), is able to induce α-MHC expression in heart cell cultures. The effects of DITPA on LV contractile performance, cardiac myosin isoenzyme composition and hepatic GPDH activity then were studied in thyroidectomized rats treated with the compound for 10 to 12 days. The results indicate that DITPA causes significantly less tachycardia and stimulation of GPDH activity than thyroxine at doses that produce near maximum inotropic action and α-MHC induction. Because heart rate is a major determinant of myocardial oxygen consumption (Boerth *et al.*, 1969), identification of a thyroid analog with sparing effects on heart rate represents progress toward the development of a useful inotropic agent.

Methods

Animals. Male Sprague-Dawley rats which had been subjected to thyroidectomy at 7 weeks of age were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Animals were maintained on rat chow (Ralston Purina Co., St. Louis, MO) and given water *ad libitum* containing methimazole (200 mg/l) and calcium chloride (130 mg/l) for 3 weeks before undergoing studies. At the end of this period, hypothyroid rats (175–200 g) were separated randomly into three T_4 treatment groups (1.5, 6.0 and 15 μg/100 g b.wt.) and three DITPA treatment groups (150, 375 and 1500 μg/100 g). Parenthetically, the dose of T_4 required to provide normal oxygen consumption and suppression of goiter formation in hypothyroid rats is approximately 1.5 μg/100 g (Stasilli *et al.*, 1959). Hypothyroid rats injected s.c. with diluent were used as controls. All animals were treated in accordance with American Association for the Accreditation of Laboratory Animal Care guidelines.

For animal experiments, stock solutions of T_4 and DITPA were prepared by dissolving the powder in 50% ethanol at pH 12. The solutions were diluted to 2 mg/ml with distilled water. Dilutions of stock solutions were made with 0.9% saline and adjusted to pH 8 to 9 before s.c. injection once daily for 10 to 12 days.

Cell culture. Primary cultures of cardiomyocytes were prepared from 18 to 19 day gestational age fetal rat heart cells by digestion with pancreatin essentially as described earlier (Nag and Cheng, 1984; Gustafson *et al.*, 1987) with minor modifications. After differential plating to remove fibroblasts and other cellular contaminants, the cells were resuspended in Dulbecco's Minimal Essential Medium containing epidermal growth factor at 10 ng/ml, penicillin at 100 U/ml, streptomycin at 100 μg/ml and the mixture of insulin (5 μg/ml)-transferrin (5 μg/ml)-selenium (5 ng/ml). Cells were plated at a density of 6.0×10^6 cells/plate onto 100-mm Petri dishes that had been coated with collagen and fibronectin. Cultures consisted of greater than 90% myocytes and within 24 hr after plating most of the cells appeared to contract. Stock solutions of T_3 or DITPA were prepared in 0.1 N NaOH and diluted in culture medium before use. The medium was changed the next day and T_3 analogs or diluent were added to the cultures.

Hemodynamics measurements. After 10 to 12 days of treatment, the animals were anesthetized with methoxyflurane and a 1-mm micromanometer-tipped catheter (Millar Instruments, Houston, TX) was inserted into the right carotid artery. The catheter was advanced into the aorta and then into the LV utilizing constant pressure monitoring. The zero pressure base line was obtained previously by placing the pressure sensor in 37°C saline. After initial recordings were obtained, the catheter was exteriorized to the midcervical region, and the animals were allowed to recover from anesthesia and surgery for at least 4 hr before conscious hemodynamic measurements were obtained. Pressure data were recorded on a physiologic recorder (model 2400, Gould Instrument, Cleveland, OH). The LV dP/dt) was obtained from a differentiating circuit in the recorder with the high-filter frequency cut-off set at 100 Hz. Three successive measurements 5 min apart were performed to assure reproducibility and averaged data are reported. After completion of all measurements, the animals were killed by decapitation. The hearts were excised quickly and washed in cold phosphate-buffered saline. The hearts were then weighed and frozen in liquid N_2 before storage at −70°C.

Myosin preparation and electrophoresis. Myosin was extracted from frozen samples of LV tissue by a modification of the method of Bhan and Scheuer (1975) as described earlier (Sheer and Morkin, 1984). The final myosin precipitate was stored in 50% glycerol at −20°C. Samples containing 0.5 μg of myosin were loaded onto sodium dodecyl sulfate-gradient gels and subjected to electrophoresis as described by Esser *et al.* (1988). The gels were fixed with 5% trichloracetic acid and stained according to the procedure of Fairbanks *et al.* (1971). After destaining, the gels were scanned with a soft laser densitometer (Biomed Instruments, Fullerton, CA) equipped with software for integration of peak areas.

RNA preparation and dot-blot assays. Total cellular RNA was isolated from ventricular muscle using the guanidinium isothiocyanate-hot phenol method (Maniatis *et al.*, 1982). All plastic and glassware were pretreated with 0.1% diethylpyrocarbonate at 37°C overnight and autoclaved. The final RNA pellet was resuspended in distilled H_2O and stored at −70°C. Levels of α- and β-MHC mRNAs were measured by use of a dot-blot assay as described earlier (Gustafson *et al.*, 1986). Individual spots were cut from the filters and the radioactivity was determined by liquid scintillation counting in Biosafe II (Research Products International, Mount Prospect, IL). Aliquots of total RNA prepared from rat liver were spotted at the same concentrations as the cardiac mRNAs. These spots were counted, and the values were considered to represent nonspecific background hybridization. All values were normalized so that maximal α-MHC induction at the largest dose of T_4 represented 100%. The average value for β-MHC mRNA in hypothyroid animals was taken as 100% β-MHC mRNA. In cell culture experiments, counts obtained with RNA from untreated plates were subtracted from counts obtained in treated plates and normalized taking values obtained with 10^{-8} M T_3 to represent 100% α-MHC mRNA induction.

GPDH assay. For assay of GPDH activity, liver mitochondrial membranes were prepared according to the procedure of Kadenbach (1966). Enzyme activity was determined by measuring the reduction of cytochrome *C* at 550 nm in a recording spectrophotometer (Gilford Instruments, Oberlin, OH). The assay mixture (1 ml) contained (in millimolar): phosphate, 100, pH 7.5; EDTA, 5; KCN, 1; L-α-glycerol phosphate, 25; cytochrome *C*, 0.09; and phenazine methosulfate, 0.1. Glycerol phosphate was omitted from the assay mixture in the reference

cuvette. The activity was expressed as micromoles of cytochrome *C* reduced per minute per milligram of protein.

Hormone binding assay. Extracts of *Escherichia coli* containing soluble human brain *c-erbA* protein (TRα_1) or human placental *c-erbA* protein (TRβ_1) prepared as described by Lin *et al.* (1990) were the kind gift of Dr. S.-Y. Cheng (National Institutes of Health, Bethesda, MD). Competition experiments were carried out by incubating bacterial extracts containing: 1 to 5 μg of protein in 50 mM Tris-HCl, pH 8.0; 0.2 M NaCl; 1 mM dithiothreitol; 0.01% Lubrol; and 20% glycerol (Buffer A) with 0.2 nM [3′-^{125}I]T_3 (2200 Ci/mmol, DuPont-New England Nuclear, Boston, MA) and increasing concentrations of unlabeled T_3 or DITPA in a final volume of 0.25 ml at 22°C for 90 min. Nonspecific binding was measured in the presence of 1000-fold excess of unlabeled ligand. Protein bound [^{125}]T_3 was separated from the unbound radioligand by filtration through nitrocellulose membranes (Schleicher-Schuell, BA85, 0.45 μm) as described by Inoue *et al.* (1983).

Data analysis. Thyroid hormone receptor binding data were analyzed with LIGAND, the logistic nonlinear curve fitting program described by Munson and Rodbard (1980). Unpaired *t* tests (Zar, 1974) were used to compare hemodynamic and biochemical results and differences were considered significant at values of $P < .05$.

Results

Effects on MHC mRNAs in cultured myocytes. The effects of T_3 and the carboxylic acid analog DITPA on α-MHC mRNA induction in cultured cardiomyocytes are shown in figure 1. Addition of T_3 to the culture medium for 48 hr caused a marked dose-dependent increase in α-MHC mRNA levels with an $EC_{50} \approx 5 \times 10^{-7}$ M. Although DITPA is thought to be relatively metabolically inert (Stasilli *et al.*, 1959; Blank *et al.*, 1966), induction of α-MHC was observed at approximately 100-times higher concentration than T_3.

Animal experiments. Results for heart weight and body weight of control rats and animals treated with T_4 and DITPA are shown in figure 2. As would be anticipated, untreated hypothyroid animals increased only very slightly in body weight (5.0 ± 1.5%) during the experiment. Administration of T_4 and DITPA caused significant increases in body weight at all dosages. At the largest dose of DITPA administered (1500 μg/100 g), there was less increase in heart weight/body weight ratio than with the largest dose of T_4 (15 μg/100 g) $P < .05$).

The effects of T_4 and DITPA on LV pressures, heart rate and LV *dP/dt* also are shown in figure 2. Although both compounds produced dose-dependent increases in these hemodynamic parameters, the potency of T_4 was two orders of magnitude greater than DITPA. The largest doses of DITPA and T_4 produced similar increases in LVSP and LV *dP/dt*, however. In the group receiving 1500 μg/100 g of DITPA, the average value LV *dP/dt* (9253 ± 796) was similar to the average value in the group receiving 15 μg/100 g of T_4 (8483 ± 350). Heart rate also was increased by both compounds. At the highest dosages administered, however, the average increase in heart rate with DITPA (67 ± 16 beats/min) was only about 55% of the average increase observed with T_4 (120 ± 17 beats/min). This difference in average heart rates between the two compounds was statistically significant ($P < .02$).

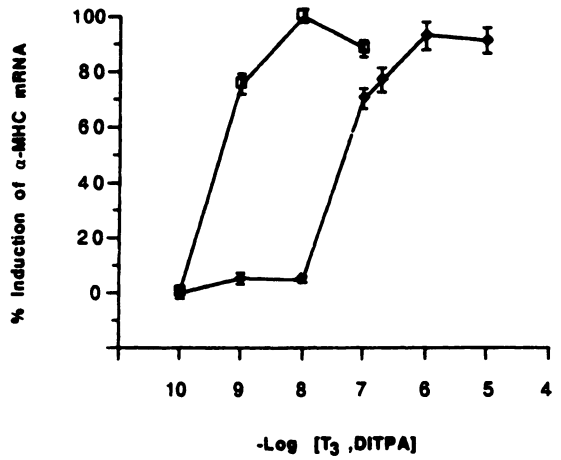

Fig. 1. Effects of thyroid hormone analogs on induction of α-MHC mRNA in cultured fetal rat heart cells. □, T_3; ◆, DITPA. Values represent means ± S.E.M. for duplicate determinations on three treated plates *vs.* three untreated control plates. In some cases, error bars are contained within the symbols.

The relatively greater inotropic than chronotropic effect of DITPA is seen more clearly by plotting LV *dP/dt vs.* heart rate (fig. 3). The curve for DITPA is displaced to the left of the T_4 curve so that for any given increase in LV *dP/dt* there is a smaller increase in heart rate with DITPA.

The effects of T_4 and DITPA on LV MHC mRNA levels and protein content are shown in figure 4. Both compounds produced switching of LV MHC content and mRNAs from a predominance of the β- to the α-MHC form. Although T_4 produced more complete switching of MHC content, the LV *dP/dt* response at any level of α-MHC content was equivalent or greater with DITPA (fig. 5).

Dose-activity relationships obtained with T_4 and DITPA for hepatic GPDH activity are shown in figure 6. Enzyme activity was increased markedly with both compounds but, as would be anticipated, T_4 was much more potent. At the highest dosage of DITPA the activity was lower than observed with T_4 ($P < .02$).

A profile of hemodynamic and metabolic effects of DITPA and T_4 at the highest dosage of each compound are shown in figure 7. Among the parameters studied, average values for LV *dP/dt*, LVSP and percentage of increase in α-MHC mRNA were not significantly different, whereas heart rate, α-MHC content and GPDH in DITPA-treated rats were less in DITPA-treated animals. Thus, DITPA was less active than T_4 on a weight basis, but its inotropic and metabolic activities were accompanied by considerably smaller increments in heart rate.

Receptor binding studies. Representative competition binding curves for T_3 and DITPA to bacterially expressed TRα_1 and TRβ_1 are shown in figure 8. Average binding affinities (K_a) of TRα_1 and TRβ_1 for T_3, which were determined from pooled data for three experiments, gave nearly the same values ($1.92 \pm 0.36 \times 10^9$ M^{-1} and $1.16 \pm 0.89 \times 10^9$ M^{-1}, mean ± S.E.M.), respectively. These values for T_3 binding are similar to those reported in whole nuclei or solubilized nuclear preparations *in vitro* (Oppenheimer, 1983). Binding studies with DITPA also failed to reveal any evidence of preferential binding to expressed receptors. The K_a of DITPA for TRα_1 was $2.40 \pm 0.77 \times 10^7$ M^{-1} and for TRβ_1 was $4.06 \pm 0.66 \times 10^7$ M^{-1}, mean ± S.E.M. These values are approximately 100-fold less than the affinity of these receptors for T_3, which is consistent with the lower biological activity of DITPA.

Discussion

The results indicate that DITPA, a thyroid analog with relatively low metabolic activity, has selective actions on the heart, stimulating contractile function and expression of α-

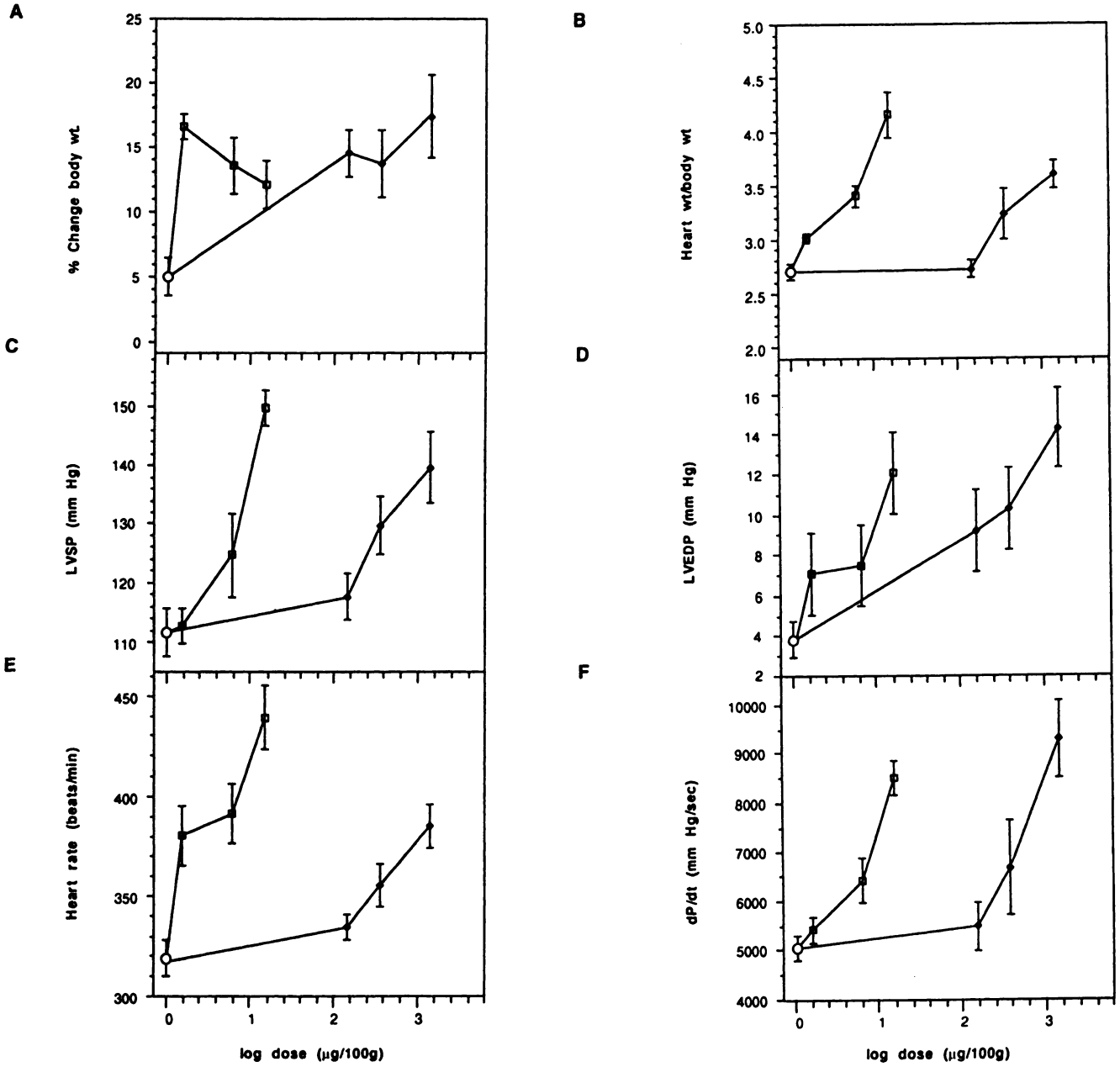

Fig. 2. Effects on hypothyroid rats of treatment with T_4 and DITPA for 10 to 12 days. □, T_4; ◆, DITPA; ○, untreated hypothyroid control rats. A percentage of change in body weight; B. heart weight/body weight ratio; C, LVSP; D, LV end-diastolic pressure (LVEDP); E, heart rate; F, LV *dP/dt*. Values represent mean ± S.E.M. for 6 to 13 animals.

MHC mRNA with significantly less chronotropic action than T_4. Several aspects of these results seem important. First, because heart rate is a major determinant of myocardial O_2 consumption, the inotropic action of DITPA presumably was accomplished at a lower myocardial O_2 cost than T_4. Secondly, the inotropic response to DITPA was higher than T_4 for any degree of α-MHC expression, indicating that additional mechanisms are important for the inotropic actions of thyroid hormone. Finally, at doses of each compound which produced equivalent inotropic responses, DITPA had less effect on general body metabolism than T_4 as judged from measurements of hepatic GPDH activity.

In the past, a number of biochemical alterations have been found in association with the inotropic actions of thyroid hormones, including induction of the high-activity ATPase V_1 (α,α) cardiac myosin isoform, stimulation of membrane Na/K-ATPase and sensitization of myocardium adenylate cyclase to the effects of catecholamines (for review see Morkin *et al.*, 1983). Accumulated evidence suggests that none of these biochemical changes represent the major underlying mechanism of the inotropic action (Dillmann, 1990). Consequently, attention has turned recently to other possibilities, particularly the effects of thyroid hormones on Ca^{++} uptake, sequestration and release. It is believed that one or more of the effects of thyroid hormone on Ca^{++} transport mechanisms might produce a positive inotropic action by increasing the resting (diastolic) calcium concentration of the sarcoplasmic reticulum (Mylotte *et al.*, 1985; Davis *et al.*, 1989). Increased Ca^{++} release during systole then would result in greater activation of the contractile apparatus (Fabiato and Fabiato, 1977).

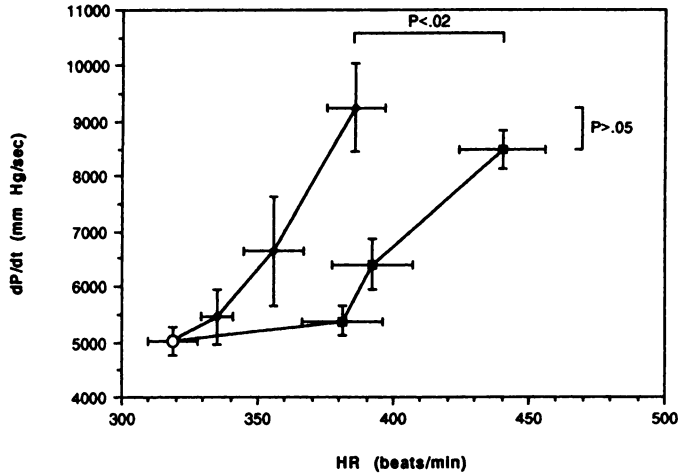

Fig. 3. Comparison of the effects of T_4 and DITPA on LV *dP/dt vs.* heart rate. □, T_4; ◆, DITPA; ○, untreated hypothyroid control rats. Values represent mean ± S.E.M. for 6 to 13 animals.

Calcium uptake and the Ca^{++}-ATPase activity of SR microsomes are reported to be increased during hyperthyroidism and decreased in hypothyroidism (Suko, 1973; Limas, 1978; Ling *et al.*, 1988). The possibility that these effects can be explained by an increase in the number of Ca^{++} pump sites in the SR is suggested by the rapid increase in the mRNA encoding SR Ca^{++}-ATPase that occurs after thyroid hormone treatment (Rohrer and Dillmann, 1988). SR Ca^{++}-ATPase mRNA levels remain elevated in hyperthyroid rats and are depressed in hypothyroid rats (Rohrer and Dillmann, 1988; Nagai *et al.*, 1989). In addition to Ca^{++}-ATPase, the expression of several other SR proteins are changed in hyperthyroidism and hypothyroidism (Arai *et al.*, 1991), suggesting that alterations in Ca^{++} uptake and release occur in response to changes in thyroid hormone levels.

The mechanism for the chronotropic action of thyroid hormone has not been studied extensively. Although it is possible that stimulation of general body metabolism may contribute to heart rate effects of thyroid hormone, earlier studies have shown direct chronotropic effects in isolated atria and perfused hearts from thyrotoxic animals (Priestly *et al.*, 1931; Yater, 1931). The hormone also has been shown to induce an increase in beating rate in cultured neonatal rat heart cells (Kessler-Icekson, 1988).

Characteristically, heart rate begins to increase between 18 and 48 hr after administration of T_3 (Brooks *et al.*, 1985). Intracellular electrode recordings indicate that the increase in heart rate is accompanied by a decrease in resting membrane potential and shortening of the duration of the action potential (Binah *et al.*, 1987; Johnson *et al.*, 1973). By contrast, in hypothyroidism the resting membrane potential is not changed and the duration of the action potential is prolonged. Because both depolarization and repolarization of the action potential are affected, it has been suggested that thyroid hormone may selectively influence transmembrane conductance. For example, an increase in Na^+ conductance relative to the decrease in K^+ conductance could explain the changes in diastolic repolarization. Details of the changes produced by thyroid hormone in the gating and kinetic properties of specific cardiac membrane ionic channels, however, require further study.

The reason for the relatively greater inotropic than chronotropic effects of DITPA is unclear, but a possible explanation might be that the responses are mediated by T_3 receptors with different affinities toward thyroid analogs. It is now established that diverse forms of the T_3 receptor are produced from the α and β *c-erbA* genes by alternative splicing (Evans, 1988). The liver seems to contain mostly the β_1 subtype, whereas in the heart the α_1 form predominates. The α_1 and β_1 receptor subtypes have been reported to have similar binding affinity for T_3 and T_4 (Murray *et al.*, 1988). Although their affinity for thyroid hormone receptor analogs has not been examined extensively, the translational products of the β_1 thyroid receptor

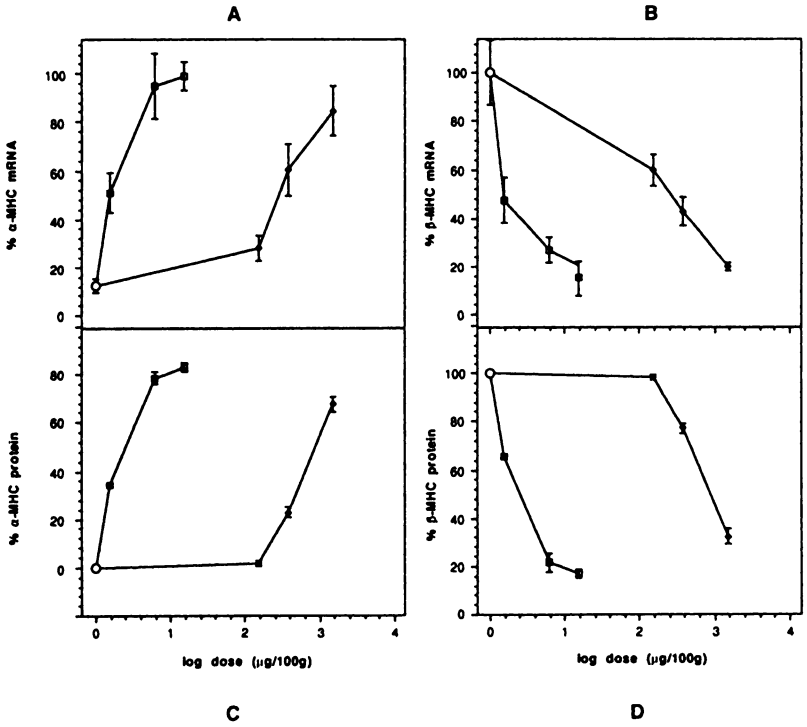

Fig. 4. Effects of T_4 and DITPA on LV content of MHCs and MHC mRNAs. □, T_4; ◆, DITPA; ○, untreated hypothyroid control rats. A, percentage of increase in α-MHC mRNA. B, percentage of decrease in β-MHC mRNA. C, percentage of increase in α-MHC protein. D, percentage of decrease in β-MHC protein. Values represent mean ± S.E.M. for 6 to 13 animals.

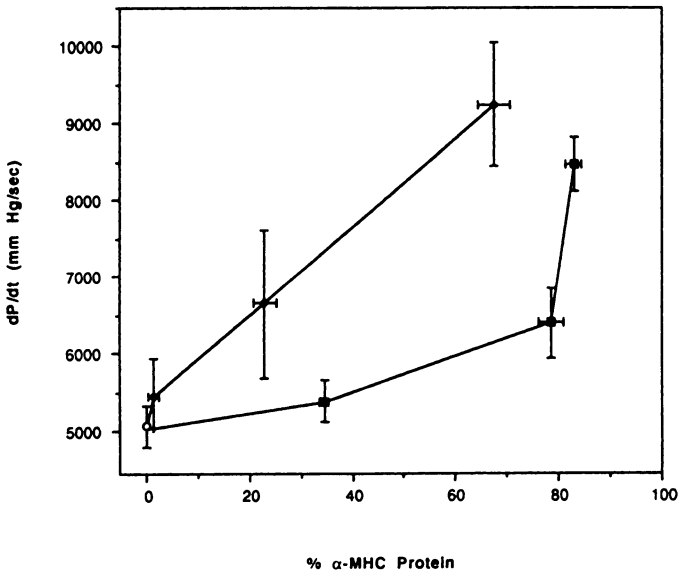

Fig. 5. Comparison of the effects of T_4 and DITPA on LV *dP/dt vs.* LV α-MHC content. □, T_4; ◆, DITPA; ○, untreated hypothyroid control rats. Values represent mean ± S.E.M. for 6 to 13 animals.

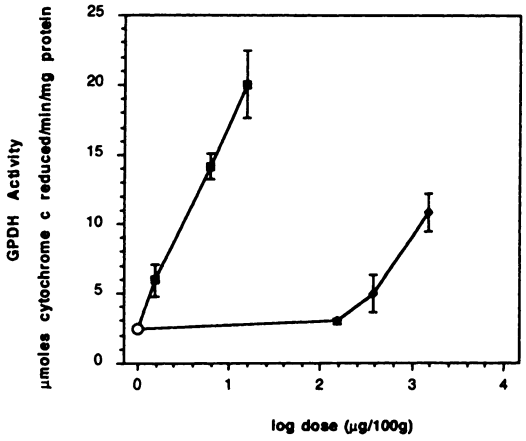

Fig. 6. Dose-activity relations of T_4 and DITPA for hepatic GPDH activity. □, T_4; ◆, DITPA; ○, untreated hypothyroid control rats. Values represent mean ± S.E.M. for 3 to 6 animals.

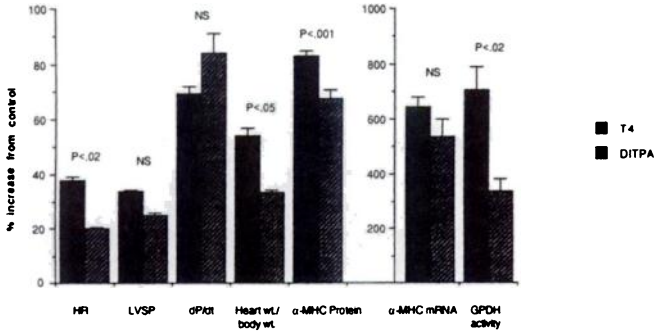

Fig. 7. Effects of largest dose of T_4 and DITPA on heart rate (HR), LVSP, LV *dP/dt*, heart weight (wt.)/body wt. ratio, α-MHC content, α-MHC mRNA and GPDH activity. Values are mean ± S.E.M. for 6 to 13 animals.

isoform have been reported recently to have a higher affinity for Triac than for T_3 (Schueler *et al.*, 1990). These observations suggested to us that DITPA, which also is a carboxylic acid derivative, might have different affinities for the receptor subtypes. Direct measurements of binding affinities of DITPA for

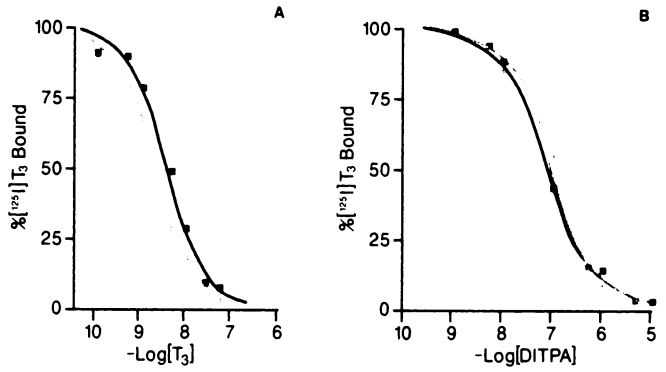

Fig. 8. Binding of bacterially expressed thyroid hormone receptors to T_3 (A) and DITPA (B). Extracts containing $TR\alpha_1$ (●) or $TR\beta_1$ (■) were incubated with 0.2 nM [^{125}I]T_3 in Buffer A (see "Materials and Methods") together with increasing concentrations of unlabeled T_3 or DITPA at 22°C for 90 min. Representative curves with each ligand are shown. Data points representing the average of triplicate determinations have been fit using a one-site nonlinear least-squares regression analysis (Munson and Rodbard, 1980). Average binding affinities (K_a) were calculated with pooled data from three experiments. The K_a values for binding of T_3 to $TR\alpha_1$ and $TR\beta_1$ were 1.92 ± 0.36 × 10^9 M^{-1} and 1.16 ± 0.89 × 10^9 M^{-1}, mean ± S.E.M., respectively. Values for binding of DITPA to the *alpha*-1 and *beta*-1 receptor subtypes were 2.40 ± 0.77 × 10^7 M^{-1} and 4.06 ± 0.66 × 10^7 M^{-1}.

bacterially expressed $TR\alpha_1$ and $TR\beta_1$ subtypes, however, failed to confirm this expectation (fig. 8). Other explanations for the differences in the effects of T_4 and DITPA on cardiac performance include greater uptake of the native hormone into pacemaker tissue than contracting muscle or differential binding to as yet undefined receptor sites. These possibilities remain to be explored.

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References

ARAI, M., OTSU, K., MACLENNAN, D. H., ALPERT, N. R. AND PERISAMY, M.: Effect of thyroid hormone on the expression of mRNA encoding sarcoplasmic reticulum proteins. Circ. Res. **69:** 266–276, 1991.

BHAN, A. AND SCHEUER, J.: Effects of physical training on cardiac myosin ATPase activity. Am. J. Physiol. **228:** 1178–1182, 1975.

BINAH, O., ARIELI, R., BECK, R., ROSEN, M. R. AND PALTI, Y.: Ventricular electrophysiology properties: Is interspecies variability related to thyroid state? Am. J. Physiol. **252:** H1265–H1274, 1987.

BLANK, B., PFEIFFER, F. R. AND GREENBERG, C. M.: Thyromimetics. VI. The synthesis and biological screening of 3,5-diiodothyroacetic and -proprionic acid analogs. J. Med. Chem. **9:** 832–834, 1966.

BOERTH, R. C., COVELL, J. W., POOL, P. E. AND ROSS, J., JR.: Increased myocardial oxygen consumption and contractile state associated with increased heart rate in dogs. Circ. Res. **24:** 725–734, 1969.

BROOKS, I., FLYNN, S. G., OWEN, D. A. A. AND UNDERWOOD, A. H.: Changes in cardiac function following administration of thyroid hormones in thyroidectomised rats: Assessment using the isolated working rat heart preparation. J. Cardiovasc. Pharmacol. **7:** 290–296, 1985.

CHENG, S.-Y., GONG, Q.-H., PARKINSON, C., ROBINSON, E., APPELLA, E., MERLINO, G. T. AND PASTAN, I.: The nucleotide sequence of a human cellular thyroid hormone binding protein present in endoplasmic reticulum. J. Biol. Chem. **262:** 11221–11227, 1987.

DAVIS, F. B., CODY, V., DAVIS, P. J., BORZYNSKI, L. J. AND BLAS, S. D.: Stimulation by thyroid hormone analogues of red blood cell Ca^{2+}-ATPase activity *in vitro*. J. Biol. Chem. **258:** 12373–12377, 1983.

DAVIS, P. J., DAVIS, F. B. AND LAWRENCE, W. D.: Thyroid hormone regulation of membrane Ca2+-ATPase activity. Endo. Res. **15:** 651–682, 1989.

DILLMANN, W. H.: Biochemical basis of thyroid hormone action in the heart. Am. J. Med. **88:** 626–630, 1990.

ESSER, K. A., BOLUYT, M. O. AND WHITE, T. P.: Separation of cardiac myosin heavy chains by gradient SDS-PAGE. Am. J. Physiol. **255:** H659–H663, 1988.

EVANS, R.: The steroid and thyroid hormone receptor superfamily. Science (Wash. DC) **240:** 889–895, 1988.

FABIATO, A. AND FABIATO, F.: Calcium release from sarcoplasmic reticulum. Circ. Res. **40:** 119–129, 1977.

FAIRBANKS, G., STECK, T. L. AND WALLACH, D. F. H.: Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry **10:** 2606–2617, 1971.

GAY, R., GUSTAFSON, T. A., GOLDMAN, S. AND MORKIN, E.: Effects of L-thyroxine in rats with chronic heart failure after healed myocardial infarction. Am. J. Physiol. **253:** H341–H346, 1987.

GUSTAFSON, T. A., BAHL, J. J., MARKHAM, B. E. AND MORKIN, E.: Hormonal regulation of myosin heavy chain gene expression in cultured rat myocytes: Analysis of effects of thyroid hormone and other cardioactive agents on mRNA expression. J. Biol. Chem. **262:** 13316–13322, 1987.

GUSTAFSON, T. A., MARKHAM, B. E. AND MORKIN, E.: Effects of thyroid hormone on α-actin and myosin heavy chain gene expression in cardiac and skeletal muscles of the rat: Measurement of mRNA content using synthetic oligonucleotide probes. Circ. Res. **59:** 194–201, 1986.

INOUE, A., YAMAKAWA, J., YUKIOKA, M. AND MORISAWA, S.: Filter binding assay procedure for thyroid hormone receptors. Anal. Biochem. **134:** 176–183, 1983.

IZUMO, S., NADAL-GINARD, B. AND MAHDAVI, V.: All members of the MHC multigene family respond to thyroid hormone in a highly tissue-specific manner. Science (Wash. DC) **231:** 597–600, 1986.

JOHNSON, P. M., FREEDBERG, A. S. AND MARSHALL, J. M.: Action of thyroid hormone on the transmembrane potentials from sinoatrial node cells and atrial muscle cells in isolated atria of the rabbit. Cardiology **58:** 273–289, 1973.

KADENBACH, B.: Der Einfluss von Thyreoidhormonen *in vivo* auf die oxidative Phosphorylierung und Enzymaktivitaten in Mitochondrien. Biochem. Z. **344:** 49–75, 1966.

KATO, H., FUKUDA, T., PARKISON, C., MCPHIE, P. AND CHENG, S.-Y.: Cytosolic thyroid hormone-binding protein is a monomer of pyruvate kinase. Proc. Natl. Acad. Sci. U.S.A. **86:** 7861–7865, 1989.

KESSLER-ICEKSON, G.: Effect of triiodothyronine on cultured neonatal rat heart cells: Beating rate, myosin subunits and CK-isozymes. J. Mol. Cardiol. **20:** 649–655, 1988.

LIMAS, C. J.: Calcium transport ATPase of cardiac sarcoplasmic reticulum in experimental hyperthyroidism. Am. J. Physiol. **235:** H745–H751, 1978.

LIN, K.-H., FUKUDA, T. AND CHENG, S.-Y.: Hormone and DNA binding activity of a purified human thyroid hormone nuclear receptor expressed in *Escherichia coli*. J. Biol. Chem. **265:** 5161–5165, 1990.

LING, E., O'BRIEN, P. J., SALERNO, T. AND IANUZZO, C. D.: Effects of different thyroid treatments on the biochemical characteristics of rabbit myocardium. Can. J. Cardiol. **4:** 301–306, 1988.

MANIATIS, T., FRITSCH, E. F. AND SAMBROOK, J.: Isolation of total cellular RNA. Molecular Cloning: A Laboratory Manual, pp. 189–195, Cold Spring Harbor Laboratory, Cold Spring Harbor, 1982.

MORKIN, E., FLINK, I. AND GOLDMAN, S.: Biochemical and physiological effects of thyroid hormone on cardiac performance. Prog. Cardiovasc. Dis. **25:** 435–464, 1983.

MUNSON, P. J. AND RODBARD, D.: A versatile computerized approach for the characterization of ligand binding data. Anal. Biochem. **107:** 220–239, 1980.

MURRAY, M., ZOLZ, N. M., MCCREARY, N. L., MACDONALD, M. J. AND TOWLE, H. C.: Isolation and characterization of rat cDNA clones for two distinct thyroid hormone receptors. J. Biol. Chem. **263:** 12770–12777, 1988.

MYLOTTE, K. M., CODY, V., DAVIS, P. J., DAVIS, F. B., BLAS, S. D. AND SCHOENL, M.: Milrinone and thyroid hormone stimulate myocardial membrane Ca^{2+}-ATPase activity and share structural homologies. Proc. Natl. Acad. Sci. U.S.A. **82:** 7974–7978, 1985.

NAG, A. C. AND CHENG, M.: Expression of myosin isoenzymes in cardiac-muscle cells in culture. Biochem. J. **221:** 21–26, 1984.

NAGAI, R., ZARAIN-HERZBERG, A., BRANDL, C. J., FUFII, J., TADA, M., MACLENNAN, D. H., ALPERT, N. R. AND PERISAMY, M.: Regulation of myocardial Ca^{2+}-ATPase and phospholamban mRNA expression in response to pressure overload and thyroid hormone. Proc. Natl. Acad. Sci. U.S.A. **86:** 2966–2970, 1989.

OPPENHEIMER, J. H.: The nuclear receptor-triiodothyronine complex: Relationship to thyroid hormone distribution, metabolism, and biological action. *In* Molecular Basis of Thyroid Hormone Action, ed. by J. H. Oppenheimer and H. H. Samuels, Academic Press, New York, 1983.

PRIESTLY, J. T. A., MARKOWITZ, J. AND MANN, F. C.: The tachycardia of experimental hyperthyroidism. Am. J. Physiol. **98:** 357–362, 1931.

ROHRER, D. AND DILLMANN, W. H.: Thyroid hormone markedly increases the mRNA coding for sarcoplasmic reticulum Ca^{2+}-ATPase in the rat heart. J. Biol. Chem. **263:** 6941–6944, 1988.

RUDINGER, A., MYLOTTE, K. M., DAVIS, P. J., DAVIS, F. B. AND BLAS, S. D.: Rabbit myocardial membrane Ca^{2+}-adenosine triphosphatase activity: Stimulation *in vitro* by thyroid hormone. Arch. Biochem. Biophys. **229:** 379–385, 1984.

SAMUELS, H. H., FORMAN, B. M., HOROWITZ, Z. D. AND YE, Z.-S.: Regulation of gene expression by thyroid hormone. J. Clin. Invest. **81:** 957–967, 1988.

SCHUELER, P. A., SCHWARTZ, H. L., STRAIT, K. A., MARIASH, C. N. AND OPPENHEIMER, J. H.: Binding of 3,5,3′-triiodothyronine (T_3) and its analogs to the *in vitro* translational products of c-*erbA* protooncogenes: Differences in the affinity of the α- and β-forms for the acetic acid analog and failure of the human testis and kidney α-2 products to bind T_3. Mol. Endocrinol. **228:** 227–234, 1990.

SHEER, D. AND MORKIN, E.: Myosin isoenzyme expression in rat ventricle: Effects of thyroid hormone analogs, catecholamines, glucocorticoids and high carbohydrate diet. J. Pharmacol. Exp. Ther. **229:** 872–879, 1984.

STASILLI, W. R., KROC, R. L. AND MELTZER, R. I.: Antigoitrogenic and calorigenic activities of thyroxine analogues in rats. Endocrinology **64:** 62–82, 1959.

STERLING, K., BRENNER, M. A. AND SAKURADA, T.: Rapid effect of triiodothyronine on the mitochondrial pathway in rat liver *in vivo*. Science (Wash. DC) **210:** 340–342, 1980.

SUKO, J.: The calcium pump of cardiac sarcoplasmic reticulum. Functional alterations at different levels of thyroid state in rabbits. J. Physiol. (Lond.) **228:** 563–582, 1973.

SWYNGHEDAUW, B.: Developmental and functional adaptation of contractile proteins in cardiac and skeletal muscles. Physiol. Rev. **66:** 710–771, 1986.

YATER, W. M.: The tachycardia, time factor, survival period and seat of action of thyroxine in the perfused hearts of thyroxinized rabbits. Am. J. Physiol. **98:** 338–343, 1931.

ZAR, J. H.: Biostatistical Analysis, Prentice-Hall, Englewood Cliffs, 1974.

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