Nongenomic Effect of Triiodothyronine on Cell Surface β-Adrenoceptors in Cultured Embryonic Cardiac Myocytes (44103)

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Abstract. **We studied the time course of cell surface β-adrenoceptors (BAR) in cardiomyocytes in response to a single triiodothyronine (T_3) (10^{-8} *M*) stimulation. An early first increase of BAR density was observed within 2 hr (+10% versus control cells, $p < 0.05$), and a plateau was maintained for 17–20 hr. This effect was followed by a much greater, late increase of BAR density, starting around 22 hr and lasting until 48 hr post T_3 addition (+40% versus control cells; $p < 0.05$). Since reverse T_3 studied in the same conditions had no effect in this system, we concluded that this T_3 effect was specific. We hypothesized that the early response might be nongenomic because the early effect of T_3 was still observed in the presence of cycloheximide (2×10^{-5} *M*) whereas the late increase was totally suppressed by the drug. The early response to T_3 required intact microtubules, since colchichine (2×10^{-5} *M*) was able to block the increase in the cell surface BAR number, but it did not involve a change in BAR distribution between external and internal sites, as the external to total BAR ratio remained stable. The measurement of the rate of BAR disappearance from the cell surface allowed us to hypothesize that T_3 induced a modification of the turnover in cell surface BAR.** [P.S.E.B.M. 1997, Vol 214]

Triiodothyronine (T_3) acts mainly through its binding to specific nuclear receptors. It results in a change in protein synthesis, explained by the modification in gene transcription due to the binding of the T_3–nuclear receptor complex to the regulatory sequence of the genes. However, some studies have also proposed extranuclear targets for thyroid hormones, such as mitochondria, Ca^{2+}-ATPase, or glucose cell transporter (for a review, see Ref. 1).

Recently, we demonstrated that T_3 could increase the β-adrenoceptor (BAR) density of chick embryonic cardiac myocytes in culture through a concentration-dependent, biphasic effect (2). The first phase of the effect was associated with a low T_3 EC_{50} (around 10^{-13} *M*), while the second was observed with an EC_{50} of 10^{-9} *M*, a value similar to the K_d of T_3 for its nuclear receptor. We postulated that the first response could be the result of a nongenomic mechanism, whereas the second could more likely be the result of the usual genomic impact. Non-genomic effects are characterized by their rapidity and their insensitivity to the blocking of protein synthesis. Few authors have evoked the possibility of rapid T_3 effects on BAR density. An early effect of T_3 (10^{-9} *M*) and T_4 (10^{-7} *M*) on cardiac BAR density was first described by Kempson *et al.* (3), who reported that thyroid hormones (TH) could increase the BAR number in rat cardiac slices in 2 hr. Since this early effect of TH was found to be insensitive to cycloheximide, they hypothesized that it involved a nongenomic mechanism. This early response to T_3 and T_4 was confirmed in the heart of hypothyroid rats, both *in vitro* and *in vivo,* by Chang *et al.* (4) and in the brain and submaxillary gland of mice by Viticchi *et al.* (5).

Moreover, other rapid cardiac effects of T_3 have been reported, such as enhanced cardiac performance within 30 min (1), increased cardiac output and stroke volume (6), and enhanced calcium uptake within 4 min in a perfused heart model (7). Rapid effects of T_3 have also been described in tissues other than the heart, by Segal (8).

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This work was supported financially by Behring-France.

Received November 1, 1995. [P.S.E.B.M. 1997, Vol 214]
Accepted October 19, 1996.

0037-9727/97/2144-0352$10.50/0

In the present study, we have evaluated the possibility of a nongenomic effect of T_3 on BAR density in cardiac myocytes in culture. We report here the existence of a first and rapidly occurring effect, followed by a second, late effect, quantitatively more important. The first effect is not inhibited in presence of cycloheximide, which strongly suggests that T_3 could increase the BAR density of cardiac myocytes in culture within 2–3 hr of T_3 stimulation *via* a nongenomic effect. This increase requires intact microtubules but does not involve any modification of BAR distribution, as described for receptor resensitization (9). The mechanism of this T_3 action probably involves a delay in the receptor spontaneous degradation. Preliminary results of this work have been presented previously (10).

Materials and Methods

Cell Culture and Treatments. Monolayer cultures of chick embryonic ventricular beating cells were prepared as previously described (2). Briefly, 11-day-old chick embryonic hearts were removed, and the ventricular fragments were dissociated by repeated cycles of incubation with porcine pancreatic trypsin, 8.5 mg/100 ml (Biosys, Compiègne, France). The resulting suspension was adjusted to 3×10^5 cells/ml in medium 199 with Hanks' salt (Eurobio, Paris, France) supplemented with 5% fetal calf serum (Gibco, Paris, France). Two milliliters of the suspension were placed in each well of six-well polystyrene culture plates. The cultures were incubated for 48 hr in a humidified 5% CO_2/95% air atmosphere at 37°C to allow for cell attachment and recovery. After 48 hr in culture, the 5% fetal calf serum medium was replaced by an insulin-containing synthetic serum-replacement medium (SSR2; Medi-Cult, Copenhagen, Denmark) with 10^{-8} *M* testosterone (Sigma, Paris, France). We did not detect protein or thyroid hormones in this synthetic medium, whereas we measured 10^{-13} *M* free T_3 in the 5% fetal calf serum medium. In contrast to the latter, this SSR2 medium prevented proliferation of the noncontractile fibroblasts. Under these conditions, more than 80% of the cells were able to contract when stimulated by an electric impulse and expressed desmin, a marker of muscular cells.

Cell membranes were prepared from cell lysate centrifugated (TRIS buffer, 10 m*M*) during 1 hr at 100 000*g* on a sucrose buffer and then washed two times with TRIS buffer.

T_3 (Sigma) and reverse T_3 (rT_3) were dissolved at 10^{-2} *M* in 26 m*M* NaOH and stored at −20°C in the dark. The stock solution was diluted with sterile distilled water to the required concentrations (10^{-8} *M*) before use. Cell treatment began 60 hr after the cell plating. Time course experiments were performed in such a way that all incubation times ended at the same time. This was made possible by the fact that the BAR number remained stable for the 72 hr of incubation in the SSR2 medium.

Cycloheximide (Sigma) was dissolved at 2×10^{-3} *M* in sterile distilled water and used at a final concentration of 2×10^{-5} *M*. To study the sensitivity of the T_3 effect to protein synthesis inhibition, cycloheximide was administered either for 12 hr (short-term effect) or 48 hr (long-term effect). For the time course experiments, cycloheximide was administered for various times alone or simultaneously with T_3. Colchicine (Sigma) was dissolved at 10^{-3} *M* in sterile distilled water, used at a final concentration of 10^{-5} *M*, and was administered 4 hr before the T_3 treatment.

Determination of BAR Density. Cell surface BAR was measured as already described (2) by using [^{3}H]CGP 12177 [−]-4-[3-tertiary-butylamino-2-hydroxypropoxy]-[5,7-^{3}H]benzimidazol-2-one, 38 Ci/mmol; Amersham, Paris, France), a BAR antagonist that cannot cross the cell membrane because of its high hydrophilic polarity. After a 48-hr incubation with different concentrations of T_3, the cells were rinsed four times with cold (4°C) Hank's balanced salt solution containing 2×10^{-5} *M* HEPES and 0.1% bovine serum albumin, pH 7.4 (HHSA). Binding was initiated by the addition of increasing concentrations (0.06–2 n*M*) of [^{3}H]CGP 12,177 in 1 ml of HHSA to the culture wells containing adherent cells. After 60 min of incubation at 37°C, the reaction was stopped by adding 2 ml of cold HHSA to each well, and the cells were rapidly washed four times with 2 ml of cold HHSA. The cells were solubilized with 1 ml 0.2 *N* NaOH, and the radioactivity of the solution was determined in a scintillation counter (Beckman, Paris, France). Nonspecific binding was defined as the amount of radioactivity remaining in the presence of 2×10^{-6} *M* (±)-propranolol, and it accounted for 5%–20% of the total binding. Binding affinity and density were calculated by Scatchard analysis. B_{max} was expressed as the number of sites per cell, and K_d was given as nanomolar.

Total cellular BAR was measured on cell membranes and on intact cells with [^{125}I]iodocyanopindolol (0–200 p*M*), a lipophilic compound that can cross the cell membrane and therefore binds to cell surface and intracellular receptors. Nonspecific binding was defined as above.

We also used [^{125}I]iodocyanopindolol to quantify the cell surface versus total BAR ratio. For that, we compared binding of [^{125}I]iodocyanopindolol in presence of (±)-propranolol (2×10^{-6} *M*), a lipophilic compound that competed with total cell sites density, and in presence of cold CGP 12177 (5×10^{-7} *M*), an hydrophilic compound that competed with [^{125}I]iodocyanopindolol for specific plasma membrane sites only.

Statistical Analysis. Data are presented as the mean ± SD or mean ± SEM, as reported in the results. One-way analysis of variance followed by the Scheffe's

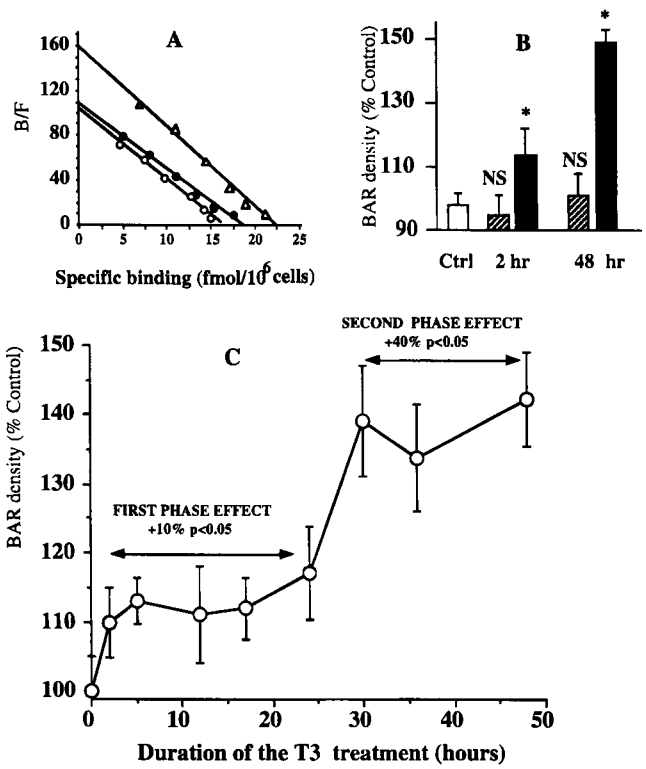

Figure 1. Time course effect of T_3 on cell surface BAR in cultured embryonic cardiac myocytes. (A) Typical scatchard plots obtained with control (○) and with T_3 (10^{-8} *M*) administered for 2 hr (●) or 48 hr (△). T_3 increases the binding site concentration without altering the dissociation constant (K_d = 0.16, 0.17, and 0.14 n*M*; and B_{max} = 16.14, 18.40, and 22.18 fmol/10^6 cells, for control, T_3 = 2 hr, and T_3 = 48 hr, respectively). (B) Comparison of the early and late effects of T_3 (▨) and rT_3 (▨). □, control value. Values are the mean ± SEM of three experiments performed in triplicate. (C) Illustration of the first- and second-phase effect of T_3. Cardiomyocytes were incubated with 10^{-8} *M* T_3 from 0 (control) to 48 hr. The number of receptors was expressed as percentage of the control value. Control cells expressed 18.4 fmol/10^6 cell. The effect of T_3 was significant ($P < 0.05$) 2 hr after T_3 addition and remained stable until 20 hr (first-phase effect). A second effect significantly different from the first-phase response was observed between 24 and 48 hr after T_3 addition. Values are the mean ± SEM of three to five experiments performed in triplicate.

test was used to compare the means of the different parameters of each group. A two-tailed test *P* value of less than 0.05 was considered to reflect a statistically significant difference.

Results

Time Course Evolution of Cell Surface BAR Cardiomyocytes in Response to T_3 Stimulation. A single concentration of T_3 10^{-8} *M* was added to the culture medium, and the density of BAR on cardiomyocytes was measured at various times up to 48 hr after T_3 addition. T_3 did not modify BAR affinity for [^{3}H]CGP 12177 (Fig. 1A). As illustrated by Figure 1C, this T_3 effect was biphasic. T_3 induced an increase of BAR density within 2 hr (+10% versus control at 2 hr; $P < 0.05$), and this level remained stable until 17 hr. This effect was defined as the first-phase effect. After 24 hr of incubation, T_3 induced a more important effect (+40% versus control at 48 hr; $P < 0.05$), defined as the second-phase, late effect of T_3. The early, first-phase effect represented about 30% of the total effect of T_3. Moreover, rT_3 had no effect on BAR density after either 5 or 48 hr of incubation (Fig. 1B).

Sensitivity to Cycloheximide of the First- and Second-Phase Effect of T_3. The BAR kinetics on cardiomyocytes in response to T_3 was measured in the presence of cycloheximide (2×10^{-5} *M*) in the culture medium. Cycloheximide altered neither the cell number nor viability. We verified that the cells remained viable and able to express spontaneous contractility even after 5 days of contact with cycloheximide where the cells were very hypotrophied.

In a first set of experiments, T_3 was administered simultaneously with cycloheximide for either 2 or 48 hr in order to compare the sensitivity of the two effects of T_3 to cycloheximide (Table I). We observed that T_3 could induce the same significant increase in BAR in 2 hr, even in the presence of cycloheximide. This result demonstrated that the first-phase effect of T_3 was insensitive to the inhibition of protein synthesis. On the contrary, the late effect of T_3 was completely abolished in the presence of cycloheximide.

We also studied the occurrence of the first-phase effect of T_3 on cells treated with cycloheximide for 12 hr. Figure 2 shows that the early effect of T_3 could be observed in these cycloheximide-treated cells. When the cells were treated with cycloheximide for 48 hr, this early effect could no longer be detected.

Effect of Colchicine on the Early BAR Increase in Response to T_3 Stimulation of Cardiomyocytes. Cells were treated with colchicine 4 hr before T_3 administration. The results illustrated in Figure 3 show that T_3 was unable to increase cell surface BAR on cardiac cells in the presence of colchichine 2×10^{-5} *M*. This finding suggested that the primary response of T_3 required intact microtubules. As the first-phase effect of T_3 was protein synthesis independent and needed intact microtubules, we hypothesized that this early effect of T_3 might involve a modification of the BAR cell repartition without any modification of the total BAR number.

Early and Late Effects of T_3 on Total BAR Number and Cell Distribution. To verify this hypothesis we tested the early and late effects of T_3 on total BAR number and cellular repartition by using [^{125}I]iodocyanopindolol, as described in Materials and Methods, on either intact cells or cell membranes. Contrary to what we expected, T_3 increased the total BAR number (Table II) and did not modify the external-to-total BAR ratio (Fig. 4). The increase in cell surface BAR was therefore the consequence of the rise of total (external and internal) BAR.

Table I. Compared Sensitivity to Cycloheximide of the Early and the Late Effect of T_3 on BAR Density

	T_3 treatment				
	None	2 hr	None	2 hr	48 hr
Cycloheximide	2 hr	2 hr	48 hr	48 hr	48 hr
B_{max}	17.36 ± 1.56	21.71 ± 1.16[a]	10.11 ± 2.6	9.80 ± 2.0	7.32 ± 1.94[b]
K_d	0.11 ± 0.052	0.11 ± 0.023	0.13 ± 0.060	0.13 ± 0.062	0.16 ± 0.032

Note. Cycloheximide (2×10^{-5} *M*) was administered simultaneously with T_3. Data are the mean ± SEM of three experiments made in triplicate.
[a]Significantly different from control value defined as cycloheximide 2 hr without T_3.
[b]Significantly different from control value defined as cycloheximide 48 hr without T_3.

Effect of T_3 on the Apparent Rate of Disappearance of BAR from the Cell Surface of Cardiomyocytes. Since BAR is a protein membrane receptor, we studied its availability at the surface of the cells while the protein synthesis was blocked. To do so, we monitored BAR density as a function of time in control cells and in cells treated with cycloheximide (2×10^{-5} *M*) for a 48-hr period. Figure 5 shows the progressive decrease in cell surface BAR density on cycloheximide-treated cardiomyocytes. This experimental system could adequately be described by the following monoexponential equation: $Y = Ae^{-kt}$, where Y was BAR density, A was the initial cell surface BAR Number (A = 12,097 sites/cell), t was the time (hr), and k (k = 0.0213) was the apparent rate constant of BAR disappearance from the surface of the cell. According to this model, the cell had to bring to the surface an average of 250 sites/hr in order to maintain a steady-state density. When the cells were treated simultaneously with T_3 and cycloheximide, the rate of disappearance of BAR from the cell surface was modified. As illustrated in Figure 5, T_3 was able to inhibit the degradation of BAR over the first 4–5 hr of treatment. This inhibition effect disappeared progressively after this period.

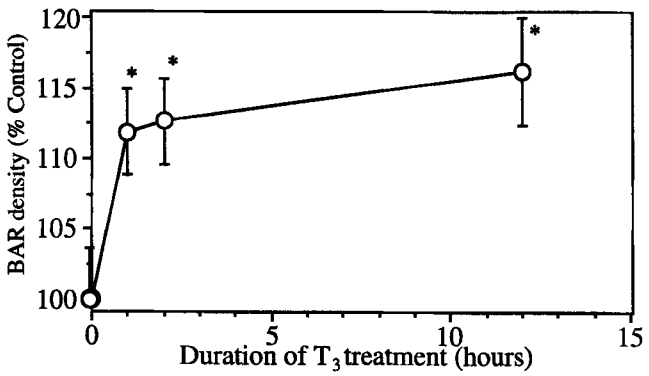

Figure 2. Time course effect of T_3 (10^{-8} *M*) on cell surface BAR number on cells treated during 12 hr with cycloheximide (2×10^{-5} *M*). A significant effect was observed for all the tested times of T_3 treatment. The number of receptors is expressed as percentage of the control value. Mean ± SEM was obtained from three experiments run with six replicates for each time.

Discussion

This study confirmed our preliminary finding (2) that T_3 (10^{-8} *M*) had two quantitatively different effects on the density of cell surface BAR in chick embryonic cardiac myocytes cultured in a protein-free medium, conditions under which the initial T_3 concentration would not be altered by proteins. An early effect could be observed within the first hours of exposure to T_3 and led to an increase in cell surface BAR density which is one third of the total effect. A second, late effect took place after 12–15 hr of treatment and gave rise to a stronger increase.

It has been reported that T_3 and T_4 used at high concentrations modify the physical properties of plasma membrane by binding very rapidly to the lipid bilayer in a nonspecific way, thus altering the accessibility of ligands to the membrane receptors (11). T_3 is the most potent thyroid hormone, T_4 being considered a prohormone. In our model, we only used T_3 in order to avoid a possible interference resulting from T_4 to T_3 conversion, which occurs in most tissue, including heart (12). However, to test the specificity of the observed effect, we

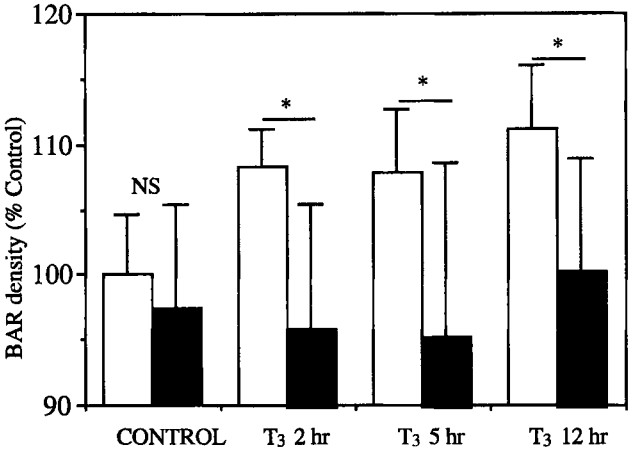

Figure 3. Comparative effect of T_3 (10^{-8} *M*) administered alone (□) or after colchicine pretreatment (■) on the number of cell surface BAR. This number is expressed as a percentage of the control value. T_3 has lost its ability to increase the density of cell surface BAR when cells were pretreated with colchicine (2×10^{-5} *M*). Values are the mean ± SD (n = 6).* $P < 0.05$. Two separate experiments giving similar results were run.

Table II. Early and Late Effects of T_3 on Total BAR Number

	Whole cells			Membranes (100,000 g)		
	Control	Early T_3 effect	Late T_3 effect	Control	Early T_3 effect	Late T_3 effect
B_{max}[a]	16.99 ± 4.34	23.70 ± 2.94[b]	33.95 ± 1.36[b]	111.2 ± 10.1	139.9 ± 7.1[c]	150.2 ± 9.8[c]
K_d (n*M*)	3.45 ± 1.0	4.6 ± 1.0	5.7 ± 0.9	18.2 ± 5.4	24.4 ± 3.3	20.5 ± 4.2

Note. Early and late effects of T_3 on total BAR number determined on whole cells and on membranes prepared as described in material and method. Data are the mean of three experiments made in triplicate.
[a] B_{max} is expressed as fmol/10^6 cells (whole cells) or fmol/mg proteins (membranes).
[b] Significantly different from whole cells control value.
[c] Significantly different from membranes control value.

added some experiments with rT_3 as control. Indeed, rT_3 had no effect on BAR density. This stereospecificity of T_3 has already been demonstrated by Kempson (3), who reported that LT_3, but not DT_3, was able to increase rapidly BAR density in rat heart slices. These results are also in agreement with those obtained *in vivo* by Szymanski (13), who reported that T_3 (after 24 hr) and T_4 (after 48 hr), but not rT_3, were able to increase BAR in the rat heart.

With cycloheximide, it was possible to demonstrate that the early increase in cell surface BAR number on cardiomyocytes in response to T_3 stimulation was not affected by this inhibitor of protein synthesis when administered for 2 and 12 hr, as already demonstrated by Kempson (3). This observation strongly supports the possibility of a nongenomic effect of T_3 on the early enhancement of BAR density of cardiomyocytes in culture. Conversely, both the early and the late responses to T_3 were markedly inhibited by cycloheximide administered for 48 hr). Cycloheximide is known to inhibit protein synthesis at concentrations ranging from 0.4 to 40×10^{-5} *M*. Since, in our conditions, cycloheximide decreased cell size and protein content (data not shown), without modifying the cell number, we can infer that protein synthesis was fully inhibited. Consequently, the early effect is independent of protein synthesis and likely requires the presence of a target whose resident life time is less than 48 hr. On the other hand, the late effect of T_3 appears to be the expression of the usual genomic effect of thyroid hormones.

To study the role of microtubules in the early effect of T_3 on BAR density, we used colchicine, which is known to inhibit microtubule polymerization and functions, such as intracellular trafficking. We have shown that this cell response to T_3 required intact microtubules. But the hypothesis that T_3 might interfere with the BAR intracellular trafficking was not confirmed by our study of BAR cell repartition. Since the T_3-induced increase we observed implied neither protein synthesis enhancement nor modification of BAR cell repartition, we have tested the alternative explanation that T_3 might slow down the degradation of BAR. In fact, T_3 was able to maintain the availability of BAR at the surface of the

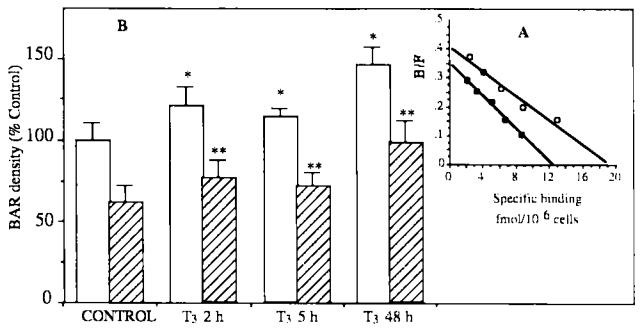

Figure 4. Effect of T_3 on BAR cell distribution. (A) Typical scatchard plots obtained with [^{125}I]iodocyanopindolol and using either propranolol or CGP 12177 for the determination of nonspecific binding as described in Material and Methods. ○, total sites; ●, external sites. (B) Effect of various incubation durations with T_3 (10^{-8} *M*) on total (□) and cell surface (▨) BAR on cultured cardiac cells. Results are expressed as a percentage of the BAR density obtained for the control value (mean of five determinations). T_3 was able to induce the same significant increase for total and cell surface BAR, consequently the external-to-total receptor ratio remained stable, being 62% ± 10%, 63 ± 11%, 63% ± 8%, and 67% ± 14%, for control, T_3 = 2 hr, 5 hr, and 48 hr respectively. $^*P < 0.05$ (versus total control); $^{**}P < 0.05$ (versus cell surface control).

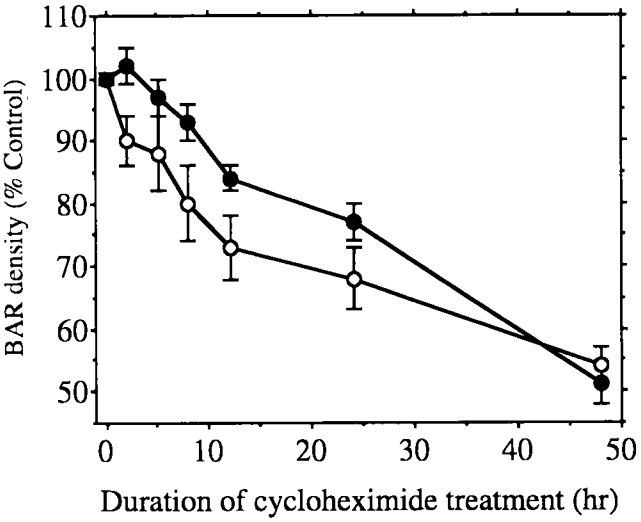

Figure 5. Comparison of the effects of cycloheximide (2×10^{-5} *M*) administered alone (○) or simultaneously with T_3 10^{-8} *M* (●) on cell surface BAR density. The numbers of receptors was expressed as a percentage of the control value and are the mean ± SD of six determinations. Similar results were obtained in three separate experiments.

cardiomyocytes by reducing its apparent rate of disappearance without changing the distribution of the receptors between external and internal sites. To our knowledge, this effect of T_3 on BAR had never been described before.

The stimulatory effect of T_3 on cardiac BAR density is very well documented. However, most studies consider the long-term effects of T_3, either *in vivo* in animal models (14) or *in vitro* in various tissue systems (15, 16). The mechanism of action by which T_3 exerts its rapid effects on BAR remains to be elucidated. Under basal conditions in intact cells, an equilibrium exists between external membrane BAR, detectable by hydrophilic ligands, and internal BAR, detectable by hydrophobic ligands. After agonist stimulation, some receptors are internalized, and the recycling of these receptors to the cell surface may contribute to their recovery. It has been hypothesized (17) that microtubules may play a critical role in the trafficking of intracellular vesicles to their eventual docking sites in the cell membrane. This need for intact microtubules is well documented for the recovery of different membrane receptors. Limas and Limas (18) demonstrated that the recovery of BAR number following a short preincubation with isoprenaline needed intact microtubules. Colchicine (10^{-5} to 10^{-4} *M*) was shown to interfere with elements of the cytoskeleton and to alter phosphorylation, which led to poor recovery of acetylcholine receptors in the membrane of snake costocutaneous muscle fibers (19). Recently, it has been proposed that BAR might be anchored to the cytoskeleton by their carboxy terminal region (20), thus affording a basis for the inhibitory effect of colchicine on BAR regulation. An example of a direct effect of thyroid hormones on a membrane-bound protein was given by Leonard *et al.* (21), who studied the regulation of type II 5′-deiodinase (5′D-II) in cultured rat glial cells. T_4 is the natural substrate of this 5′D-II, and these authors demonstrated that the biological half-life of the enzyme was T_4 dependent and that this regulation was mediated by an energy-dependent process requiring an intact actin cytoskeleton. Later, the same authors better characterized this effect by showing that T_4 could markedly increase the turnover of 5′D-II by promoting its binding to F-actin, which led to the internalization of this complex and its trafficking toward an endosomal pool (22). As such, this regulation shows some similarities to our results, even if in that particular case thyroid hormone decreased the turnover of membrane component and implicate microfilament network.

The fact that in our model the early effect of T_3 was inhibited by colchicine suggested that this T_3 effect involved a modification of BAR distribution. To verify this hypothesis, we studied the early effect of T_3 on the total cell BAR number and distribution. We found that the early effect of T_3 involved an increase in total cell number without affecting the ratio of external-to-total BAR sites. Our initial hypothesis was therefore not confirmed. It should be however noted that an *in vivo* study demonstrated that hyperthyroidism could induce an enlargement of the cardiac membrane-receptor compartment but not of the intracellular vesicular compartment (23). However, this effect was observed after 6 days of thyroid hormone administration, a lapse of time much longer than the few hours of our study. Moreover, the possible responses of the whole heart *in vivo* to T_3 are probably multifactorial, making it difficult to extrapolate to the possible *in vitro* response of cultured myocytes.

Another possibility is a direct effect of T_3 on the metabolism or the stability of BAR. It is widely accepted that adrenergic receptors have a slow turnover rate (24). In the presence of T_3, this apparent rate constant was slowed within the first hours of T_3 treatment but not after longer periods of time (30–48 hr). This protective effect on membrane BAR, observed rapidly after T_3 administration, is in accordance with recent hypothesis proposed by Canavan *et al.* (25). These authors studied the influence of thyroid hormones on the growth of heart atria and ventricles in immature rats and reported that hyperthyroidism could both increase the rate of protein synthesis and decrease the rate of protein degradation. It is important to stress the fact that thyroid hormones' effects on the heart are very specific and opposite to what is usually described in other organs in terms of protein degradation. The early increase in BAR cell surface density induced by T_3 results therefore from an increase in the stability of BAR. The mechanism of this effect is unknown but might involve post-translational modifications. It has been described that T_3 might bind to p55, a protein which is a subunit of the protein disulfide isomerase involved in post-translational modification of secretory and membrane-associated proteins and might as such be a key component in the early effect of T_3 (26).

In conclusion, the nongenomic T_3 effect on membrane BAR density in cardiac cells we described here might contribute *in vivo* to the acute cardiac inotropic effects of T_3, together with the increased influx of calcium, glucose, and amino acids, as previously reported. It could constitute the basis of further research on the genomic and nongenomic mechanisms of action of T_3 on the heart as a model for the development of new inotropic agents.

The authors would like to thank D. Anger and C. Delrieu for technical assistance and Dr. J. Jacobson for helpful advice and manuscript preparation.

1. Davis PJ. Acute cellular actions of thyroid hormone and myocardial function. Ann Thorac Surg **56:**S16–S23, 1993.
2. Yin Y, Vassy R, Nicolas P, Perret GY, Laurent S. Antagonism between T3 and amiodarone on the contractility and the density of

beta-adrenoceptors of chicken cardiac myocyte. Eur J Pharmacol **261:**97–104, 1994.
3. Kempson S, Marinetti GV, Shaw A. Hormone action at the membrane level. VII. Stimulation of dihydroalprenolol binding to beta-adrenergic receptors in isolated rat heart ventricle slices by triiodothyronine and thyroxine. Biochem Biophys Acta **540:**320–329, 1978.
4. Chang H, Kunos G. Short term effect of triiodothyronine on rat heart adrenoceptors. Biochem Biophys Res Commun **100:**313–320, 1981.
5. Viticchi C, Fattoretti P, Piantanelli L. Very rapid regulation of mouse beta-adrenoceptor density by thyroid hormones. Med Sci Res **16:**239–1240, 1988.
6. Ojamaa K, Balkman C, Klein IL. Acute effects of triiodothyronine on arterial smooth muscle cells. Ann Thorac Surg **56:**S61–S67, 1993.
7. Gotzsche LBH. L-Triiodothyronine acutely increases Ca^{2+} uptake in the isolated, perfused rat heart—Changes in L-type Ca^{2+} channels and beta-receptors during short- and long-term hyper- and hypothyroidism. Eur J Endocrinol **130:**171–179, 1994.
8. Segal J. Biphasic stimulation of cellular calcium concentration by 3,5,3′-triiodothyronine in rat thymocytes. Biochemistry **27:**2568–2590, 1988.
9. Marsh JD, Barry WH, Smith TW. Desensitization to the inotropic effect of isoproterenol in cultured ventricular cells. J Pharmacol Exp Ther **223:**60–67, 1982.
10. Vassy R, Yin YL, Perret GY. Acute effect of T3 on beta-adrenoceptors of cultured chick cardiac myocytes. In: Braverman LE, Eber O, Langsteger W, Eds. Heart and Thyroid. Graz: Blackwell-MZV, pp 165–168, 1994.
11. Farias RN, Chehin RN, Rintoul MR, Morero RD. Differential effect of Triiodothyronine and thyroxine on the liposomal membrane in liquid-crystalline and gel state. J Membr Biol **143:**135–141, 1995.
12. Sterling K. Thyroid hormones take action at the cellular level. N Engl J Med **300:**117–123, 1979.
13. Szymanski PT, Nauman J. Effect of thyroid hormones and reverse triiodothyronine (rT3) pretreatment on beta-adrenoceptors in rat heart. Acta Physiol Pol **37:**131–138, 1986.
14. Limas C, Limas CJ. Influence of thyroid status on intracellular distribution of cardiac adrenoceptors. Circ Res **61:**824–828, 1987.
15. Kim D, Smith TW, Marsh JD. Effect of thyroid hormone on slow calcium channel function in cultured chick ventricular cells. J Clin Invest **80:**88–94, 1987.
16. Karliner JS, Simpson PC. β-Adrenoceptor and adenylate cyclase regulation in cardiac myocyte growth. Basic Res Cardiol **16:**1239–1240, 1988.
17. Mill JW, Mandel LJ. Cytoskeleton regulation of membrane transport events. FASEB J **8:**1161–1165, 1995.
18. Limas CJ, Limas C. Rapid recovery of cardiac beta-adrenergic receptors after isoproterenol-induced "down"-regulation. Circ Res **55:**524–531, 1984.
19. Hardwick JC, Parsons RL. Requirement of a colchicine-sensitive component of the cytoskeleton for acetylcholine receptors recovery. Br J Pharmacol **114:**442–446, 1995.
20. Parker EM, Swigart P, Nunnally MH, Perkins JP, Ross EM. Carboxyl-terminal domains in the avian beta(1)-adrenergic receptor that regulate agonist-promoted endocytosis. J Biol Chem **270:**6482–6487, 1995.
21. Leonard JL, Siegrist-Kaiser CA, Zuckerman CJ. Regulation of type II iodothyronine 5′ deiodinase by thyroid hormone. J Biol Chem **265:**940–946, 1990.
22. Farwell AP, DiBenetto DJ, Leonard JL. Thyroxine targets different pathways of internalization of type II iodothyronine 5′-deiodinase in astrocytes. J Biol Chem **268:**5055–5062, 1993.
23. Limas C, Limas CJ. Influence of thyroid status on intracellular distribution of cardiac adrenoceptors. Circ Res **61:**824–828, 1987.
24. Mahan LC. Metabolism of alpha and beta-adrenergic receptor in vitro and in vivo. Annu Rev Pharmacol Toxicol **27:**215–235, 1987.
25. Canavan JP, Holt J, Goldspink DF. The influence of thyroid hormones on the growth of the atria and ventricles of the heart in immature rats. J Endocrinol **142:**171–179, 1994.
26. Safran M, Farwell AP, Leonard JL. Thyroid hormone-dependent redistribution of the 55-kilodalton monomer of protein disulfide isomerase in cultured glial cells. Endocrinology **131:**2413–2418, 1992.