Regulation of Type II Iodothyronine 5'-Deiodinase by Thyroid Hormone

INHIBITION OF ACTIN POLYMERIZATION BLOCKS ENZYME INACTIVATION IN cAMP-STIMULATED GLIAL CELLS*

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The cellular events mediating the rapid, thyroid hormone-dependent modulation of membrane-bound, type II iodothyronine 5'-deiodinase were studied in dibutyryl cyclic AMP (b2cAMP)-treated brain astrocytes. Unstimulated cells had undetectable type II 5'-deiodinating activity. Treating the cells with b2cAMP and hydrocortisone induced enzyme expression by increasing transcription of the enzyme or an essential enzyme related protein(s), with steady-state levels of type II 5'-deiodinase attained after 8 h of b2cAMP treatment. Glial cells grown in the absence of thyroid hormone had 10-15-fold higher levels of 5'-deiodinating activity than cells grown in the presence of serum. The increased type II 5'-deiodinating activity observed in serum-free cultures was due to a prolonged enzyme half-life with no change in the rate of enzyme synthesis. Addition of thyroxine or 3,3',5'-triiodothyronine to the serum-free culture medium resulted in a concentration-dependent fall in steady-state enzyme levels, with EC50 values of ~0.4 nm. 3,3',5'-Triiodothyronine was at least 100-fold less effective. Chloroquine, NH4Cl, tunicamycin, colchicine, and monodansylcadaverine had no effect on the T3 of the enzyme, while both carbonyl cyanide m-chlorophenylhydrazone and cytochalasins completely blocked the inactivation of the type II 5'-deiodinase. These data indicate that in glial cells, an intact actin-cytoskeleton is required for thyroid hormone to modulate the energy-dependent regulation of the half-life of the short-lived, membrane-bound enzyme, type II 5'-deiodinase.

Thyroid hormone metabolism plays a fundamental role in determining the intracellular levels of bio-active 3,5,3'-triiodothyronine (T3) in the brain. Recent studies have shown that >90% of the T3 bound to brain cell nuclei is derived from intracellular thyroxine (T4) to T3 conversion, rather than from circulating T3 (1–3). The key enzyme in this pathway is type II iodothyronine 5'-deiodinase (5'D-II) (for review see Ref. 1).

Type II 5'-deiodinase is a short-lived, integral membrane protein associated with neurolemmal membranes (4). Interestingly, cerebrocortical enzyme activity fluctuates with changes in thyroid status, increasing 5-10-fold 1 day after thyroidectomy and decreasing ~90% within 30 min of hormone replacement (5, 6). These dynamic, adaptive changes in 5'-deiodinase serve to maintain near-normal intracellular T3 levels in the brains of neonatally hypothyroid rats given only 1/10 the daily replacement dose of T4 (7), indicating that the brain has regulatory pathways that monitor and defend intracerebral T3 levels.

Recent studies in rats have shown that the thyroid hormone-dependent changes in type II 5'-deiodinase are mediated by regulation of enzyme inactivation rather than enzyme synthesis (8, 9). The demonstration that enzyme levels are more sensitive to T3 than to T4 (7, 10), together with the observation that this action of thyroid hormone is not blocked by inhibitors of transcription or translation (8), suggests a novel, extranuclear site of action for thyroid hormone in the brain.

The cellular mechanism for this thyroid hormone response has been difficult to study in intact animals, and cell culture models expressing thyroid hormone metabolizing enzymes such as dispersed fetal (11) or neonatal rat brain (12, 13), and the neuroblastoma cell line NB41A3 (14), suffer from cell heterogeneity and/or low 5'-deiodinase levels. Our demonstration that cAMP induces abundant type II 5'-deiodinase in cultured astrocytes (15) has been exploited to examine the T4-dependent regulation of this short-lived, membrane-bound enzyme. The data show that T4 and T3 rapidly modulate the rate of enzyme inactivation in these cells, whereas T3 is a much less effective hormone. In addition, inactivation and/or degradation of this membrane-bound enzyme was found to be energy dependent and to depend on the structural integrity of the actin-cytoskeleton.

MATERIALS AND METHODS

Dulbecco's modified Eagle's medium (DMEM), antibiotics, Hank's salt solution, glucose, and trypsin were obtained from Gibco. Fetal bovine serum (heat-inactivated) was obtained from Hyclone, Inc. Culture flasks and plasticware were purchased from MA Bioproducts. L-T4 and L-T3 were from Sigma; L-T4 was from Behring Diagnostics and L-3,3'-diiodothyronine was from Henning GmbH. B2cAMP, hydrocortisone, insulin, actinomycin D, prostaglandin P20, and puromycin were purchased from Sigma. Fibroblast growth factor was obtained from Collaborative Research Inc. Na125I (~17 Ci/mg) was purchased from Du Pont-New England Nuclear. L-[3' or 5']-3H]T4 (~2200 Ci/mmol) was prepared by the method of Weeke and Orskov.
phenol blue, denatured by heating in a boiling water bath for 10 min, (data not shown), an intermediate filament characteristic of showed a doubling time of 25 + 2 h that was unaffected by 1 dispersed with occasional clusters of arborized cells. Greater mM bt,cAMP for up to 48 h. (Fig. 1). Bt,cAMP was required to maintain steady-state levels deiodinase activity that plateaued after 8 h of stimulation of type II 5'-deiodinase, since removal of the cyclic nucleotide resulting in the progressive loss of the enzyme after a 60-min lag period. In subsequent studies, bt,cAMP was present with Coomassie Blue (R-250). Filamentous actin (F-actin; M, 43,000 characteristic insolubility in detergents. was measured by the method of Hill and Whatley (25). (monomer)) was identified by its electrophoretic mobility and its and separated by electrophoresis on 12.5% sodium dodecyl sulfate-pose of 50

RESULTS
Cell monolayers consisted of flat polygonal cells interspersed with occasional clusters of arborized cells. Greater than 95% of the cells contained glial fibrillary acidic protein (data not shown), an intermediate filament characteristic of astrocytes (26). Glial cells, under our culture conditions, showed a doubling time of 25 ± 2 h that was unaffected by 1 mM bt,cAMP for up to 48 h. In agreement with earlier work (19), bt,cAMP treatment resulted in the time-dependent appearance of type II 5'-deiodinase activity that plateaued after 8 h of stimulation (Fig. 1). Bt,cAMP was required to maintain steady-state levels of type II 5'-deiodinase, since removal of the cyclic nucleotide resulted in the progressive loss of the enzyme after a 60-min lag period. In subsequent studies, bt,cAMP was present throughout the experimental period. Effects of Thyroid Hormone on Type II 5'-Deiodinase in Bt,cAMP-stimulated Gial Cells—The effects of T4, T3, and rT3 on steady-state type II iodothyronine 5'-deiodinase levels are shown in Fig. 2. Cells grown in hypothyroid medium showed 10-15-fold higher steady-state levels of 5'-deiodinating activity than cells grown in euthyroid medium. Addition of increasing amounts of either T4 or rT3 resulted in the concentration-dependent fall in steady-state enzyme levels with an ED$_{50}$ of 0.4 nM or ~60 pm "free" hormone (determined by equilibrium dialysis, Fig. 2). T3 was at least 100-fold less effective in modulating steady-state enzyme levels. Effects of Individual Growth Factors on Bt,cAMP Induction of Type II 5'-Deiodinase—To examine whether one or more of the compounds in the chemically defined medium contributed to the increased expression of type II 5'-deiodinase, components were individually examined for their ability to influence 5'-deiodinating activity. In the absence of bt,cAMP, none of the compounds tested induced type II 5'-deiodinase (data not shown). Data in Table I show that fibroblast growth factor, putrescine, and prostaglandin F$_{2a}$ also had no effect on bt,cAMP-induced enzyme activity. In contrast, hydrocortisone increased 5'-deiodinating activity ~2-fold, whereas insulin depressed enzyme levels ~40%. Hydrocortisone stimulation of type II 5'-deiodinase was concentration dependent (Fig. 3A) with an ED$_{50}$ of 4 nM. As shown in Fig. 3B, the fractional disappearance of 5' deiodinating activity in cycloheximide-blocked, bt,cAMP-stimulated cells was unchanged by hydrocortisone, suggesting that the glucocorticoid-dependent increase in type II 5'-deiodinase levels was most likely due to increased enzyme production. The effect of actinomycin D on enzyme induction by bt,cAMP is shown in Fig. 4. At the start of the experiment, 1 mM bt,cAMP was added to the medium of all cells followed, at 30 min intervals, by addition of 10 µM actinomycin D. Actinomycin D-blocked cells were incubated for an additional 60 min and 5'-deiodinating activity determined. Type II 5'-deiodinase activity was undetectable for the first 45-60 min
of btzCAmp treatment and increased linearly thereafter. At the earliest time point (30 min), inhibition of RNA synthesis blocked the subsequent appearance of type II 5'-deiodinase activity, while after 60 min of btzCAmp treatment, sufficient new mRNA had been made to result in a significant increase in enzyme, at a rate ~15% of that seen in the absence of actinomycin D. The rate of enzyme accumulation in actinomycin D-blocked cells increased progressively to control levels between 60–90 min of stimulation, indicating that mRNA synthesis was no longer rate-limiting for type II 5'-deiodinase production.

Shown in Table II is the effect of actinomycin D on hydrocortisone amplification of type II 5'-deiodinase in stimulated glial cells. In btzCAmp-stimulated cells expressing steady-state levels of type II 5'-deiodinase, 40 nM hydrocortisone induced further accumulation of type II 5'-deiodinase, and actinomycin D abolished the glucocorticoid-induced increase in enzyme.

Effects of Thyroid Status on the Turnover of Type II 5'-Deiodinase—The influence of thyroid hormone on the t½ of type II 5'-deiodinase is shown in Fig. 5. Cycloheximide-dependent inhibition of protein synthesis in cells grown in euthyroid medium resulted in the exponential disappearance of type II 5'-deiodinase with a t½ of 20 min, while the enzyme in cells grown in hypothyroid medium showed a t½ of 300 min. Enzyme production rates, calculated from steady-state enzyme levels and the disappearance rate constant (k), were essentially the same in euthyroid and hypothyroid media indicating that thyroid hormone had little or no effect on enzyme synthesis (Table III). The data in Table III also show a modest reduction in btzCAmp-inducible type II 5'-deiodinase in cells after prolonged culture (i.e. passage 6). This decrement in enzyme activity was routinely observed and was progressive so that after passage 8, inducible type II 5'-deiodinase activity was only 20% of that observed at passage 2 (data not shown). For this reason, experiments were done on cells between the 2nd and 6th passage.

Effects of Antimetabolites and Cytoskeletal Inhibitors on the Turnover of Type II 5'-Deiodinase—The clearance of many integral membrane proteins is often initiated by internalization, followed by delivery to lysosomes. Since the degradation pathway for type II 5'-deiodinase was unknown, we examined the energy dependence, cell structural requirements, and role of lysosomes in the turnover of this short-lived enzyme. BtzCAmp-stimulated cells, at steady state with respect to type II 5'-deiodinase, were exposed to selected inhibitors in the presence or absence of cycloheximide and incubated for 30 min at 37 °C. As shown in Table IV, cycloheximide reduced enzyme activity by 78% at 30 min and 91% at 60 min. ATP depletion by addition of 20 μM carbonyl cyanide m-chlorophenylhydrazone, resulted in a 45% decrease in type II 5'-deiodinase, and cycloheximide did not further decrease 5'-deiodinating activity in carbonyl cyanide m-chlorophenylhydrazone-treated cells. Lysosomotropic agents (chloroquine and NH4Cl), inhibitors of endocytosis (monodansylcadaverine) or glycooxidation (tunicamycin), and microtubule disruption (colchicine) had little or no effect on enzyme levels either in the absence or presence of cycloheximide. On the other hand, colchicine rapidly reversed the btzCAmp-stimulated contraction of the glial cell borders, so that within 30 min the morphology of the btzCAmp-treated cell was indistinguishable from that of unstimulated controls (data not shown). In

| Individual component added | Type II 5'-deiodinase activity % of control |
|---------------------------|------------------------------------------|
| A. DMEM                   | - 10% fetal bovine serum 100 ± 6          |
| B. DMEM                   | - serum free 1150 ± 120a                  |
| C. + PGF2α (500 ng/ml)    | 980 ± 30                                 |
| D. + fibroblast growth factor (100 μg/ml) | 1060 ± 45 |
| E. + putrescine (100 nM)  | 930 ± 70                                 |
| F. + insulin (90 μg/ml)   | 680 ± 60a                                |
| G. + hydrocortisone (50 nM) | 2190 ± 120a |

*p < 0.01 B versus A; G versus B.

*p < 0.05 F versus B.

**Deiodinase**—The influence of thyroid hormone on the t½ of type II 5'-deiodinase is shown in Fig. 5. Cycloheximide-dependent inhibition of protein synthesis in cells grown in euthyroid medium resulted in the exponential disappearance of type II 5'-deiodinase with a t½ of 20 min, while the enzyme in cells grown in hypothyroid medium showed a t½ of 300 min. Enzyme production rates, calculated from steady-state enzyme levels and the disappearance rate constant (k), were essentially the same in euthyroid and hypothyroid media indicating that thyroid hormone had little or no effect on enzyme synthesis (Table III). The data in Table III also show a modest reduction in btzCAmp-inducible type II 5'-deiodinase in cells after prolonged culture (i.e. passage 6). This decrement in enzyme activity was routinely observed and was progressive so that after passage 8, inducible type II 5'-deiodinating activity was only 20% of that observed at passage 2 (data not shown). For this reason, experiments were done on cells between the 2nd and 6th passage.
FIG. 4. Effects of actinomycin D on appearance of type II 5'-deiodinase (5'-D-II) activity in btcAMP-stimulated glial cells. Confluent monolayers of glial cells were grown in DMEM containing 10% fetal bovine serum and antibiotics. At the start of the experiment, all flasks received btcAMP (final concentration, 1 mM) and at the times indicated by arrows, triplicate flasks received actinomycin D (final concentration, 10 nM) and the cultures then incubated for an additional 60 min at 37 °C. Cells in individual flasks were harvested by scraping and type II 5'-deiodinase determined in triplicate as described under "Materials and Methods." Data are reported as the means of closely agreeing (∓10%) triplicate cultures. (□□□) control cultures without actinomycin D; (□□□) cultures treated with actinomycin D.

TABLE II

| Treatment conditions | Time | Type II 5'-deiodinase activity |
|----------------------|------|-------------------------------|
|                      | min  | Control | Actinomycin D |
| No additions         | 30   | 620 ± 25 | 634 ± 29 |
|                      | 60   | 769 ± 40 | 645 ± 84 |
| 100 nM hydrocortisone| 120  | 984 ± 126 | 610 ± 17 |

Fig. 3. Effects of hydrocortisone on induction of type II 5'-deiodinase (5'-D-II) activity in btcAMP-stimulated glial cells. A, triplicate flasks (25 cm²) of confluent glial cells were treated with 1 mM btcAMP for 16 h at 37 °C in serum-free DMEM containing antibiotics and increasing concentrations of hydrocortisone as indicated. Enzyme activity was determined in cell sonicates as described under "Materials and Methods." Data are reported as the means of closely agreeing (∓10%) replicates. B, triplicate flasks (25 cm²) of cells were treated for 16 h with 1 mM btcAMP in DMEM containing 10% fetal bovine serum and antibiotics, ±100 nM hydrocortisone. At the start of the experiment, cycloheximide was added to a final concentration of 100 μM and the cells were incubated at 37 °C for the times indicated. Enzyme activity was determined in duplicate on cell sonicates from individual flasks as described under "Materials and Methods." Data are reported as the mean of closely agreeing (∓10%) replicates.

In contrast, cytochalasin B and the more selective microfilament inhibitor, dihydrocytochalasin B, preserved ~75% of the type II 5'-deiodinase in the cycloheximide-blocked cell.

Shown in Fig. 6 is the effect of 10 μM cytochalasin B on the t₀ of type II 5'-deiodinase. In the presence of cytochalasin B and cycloheximide, type II 5'-deiodinase activity progressively disappeared for the first 10 min. Thereafter, enzyme activity remained nearly constant in cytochalasin B-treated cultures, while 5'-deiodinating activity continued to decrease exponentially in cycloheximide-blocked cells. Pretreatment with cytochalasin B for 15 min prior to cycloheximide treatment abolished the rapid first phase of loss of type II 5'-deiodinase suggesting that this initial phase occurred during a period when the actin polymerization was not completely inactivated (data not shown). Analysis of the effects of cytochalasin B on the actin-cytoskeleton showed that filamentous actin in cytochalasin B-treated cells decreased by 25% after 5 min and 43% after 15 min, as judged by densitometric analysis of the 43-kDa actin band present in the triton-insoluble cytoskeleton after sodium dodecyl sulfate-polyacrylamide gel electrophoresis. These data suggest that ~15 min of cytochalasin treatment is required to inhibit actin polymerization sufficiently to decrease the cellular filamentous actin content to levels that impair the inactivation of type II iodothyronine 5'-deiodinase.

In the absence of cycloheximide, cytochalasins promoted the further accumulation of type II 5'-deiodinase at a rate (1.7-2.4%/min) 60-80% of the estimated enzyme production rate (3%/min; (the product of type II iodothyronine 5'-deiodinase activity at steady-state (100%) x k)) (Table IV) indicating that depolymerization of the actin-cytoskeleton selectively blocked the loss of type II 5'-deiodinase without altering the synthetic pathway for the integral membrane enzyme.
cerebral cortex of hypothyroid rats (5,7), are readily achieved deiodinase in the central nervous system. Steady-state levels concentration) for an additional 16 h. Cycloheximide (0.1 DMEM (M) for 24 h and stimulated with 1 mM bt,cAMP (final protein and 1230 ± 63 (SD.) units/mg protein in the presence or absence of serum, respectively. Type II 5'-deiodinase activity was measured as described under "Materials and Methods." Results are expressed as the mean percentage ± S.D. of the remaining enzyme activity from eight experiments for euthyroid and three experiments for hypothyroid cells. Steady-state enzyme levels were 84 ± 9 (S.D.) units/mg protein and 1230 ± 63 (S.D.) units/mg protein in the presence or absence of serum, respectively.

**TABLE III**
Effects of thyroid hormone on calculated synthesis rates of type II 5'-deiodinase in bt,cAMP-stimulated glial cells

Cells were grown in euthyroid (DMEM/10% fetal bovine serum (FBS) or hypothyroid (chemically defined, CD-serum) medium for 5 days and treated with 1 mM bt,cAMP for 16 h to induce steady-state levels of type II 5'-deiodinase. Enzyme activity was determined in cell sonicates as described under "Materials and Methods" and reported as units, where 1 unit equals 1 fmol I- released.min-1 mg protein-1. Fractional disappearance of the enzyme was determined as described in legend to Fig. 5. Production rate = steady-state enzyme activity (units) X fractional turnover (h-1).

| Passage no. | Culture conditions previous 5 days | Fractional turnover | Enzyme activity | Production rate |
|-------------|-----------------------------------|---------------------|-----------------|----------------|
| 4           | DMEM/10% FBS CD-serum free         | h-1                 | units           | unit/h         |
| 6           | DMEM/10% FBS CD-serum free         | 3.8                 | 57              | 211            |

**DISCUSSION**

In this study, we have shown that the bt,cAMP-stimulated glial cell is an excellent model system for the characterization of the molecular events regulating the turnover of type II 5'-deiodinase in the central nervous system. Steady-state levels of type II 5'-deiodinase, 50-100 times those observed in the cerebral cortex of hypothyroid rats (5,7), are readily achieved within 8 h of stimulation with bt,cAMP (15,27) and are maintained for up to 24 h in the presence of the cyclic nucleotide. Cellular differentiation does not appear to mediate the induction of type II 5'-deiodinase, since bt,cAMP did not slow the growth rate of the cultured astrocytes and previous work has shown that butyrate, a differentiating agent (28), did not induce type II 5'-deiodinase (15,27).

The appearance of type II 5'-deiodinating activity stimulated by cyclic nucleotides was preceded by a transcriptional event(s) and synthesis of new mRNA encoding either the enzyme or an essential enzyme regulatory protein quickly reached non-rate limiting levels. Interestingly, enzyme induction was amplified by hydrocortisone by a mechanism requiring increasing transcription. However, the glucocorticoid effect was only seen in the presence of bt,cAMP indicating an absolute requirement for cyclic nucleotide for the expression of type II 5'-deiodinase in the cultured astrocyte. Thyroid hormone had a marked influence on glial cell type II 5'-deiodinase with 10-20 fold increases in activity achieved in cells grown in the absence of thyroid hormone, similar to

**Fig. 5. Effects of cycloheximide on type II 5'-deiodinase (5',D-II) activity in bt,cAMP-stimulated glial cells.**

Cultures were grown in supplemented DMEM (■) or serum-free DMEM (□) for 24 h and stimulated with 1 mM bt,cAMP (final concentration) for an additional 16 h. Cycloheximide (0.1 mM) was added, and the triplicate flasks of cells (25 cm2) were harvested at the times indicated. Type II 5'-deiodinase activity was measured as described under "Materials and Methods." Results are expressed as the mean percentage ± S.D. of the remaining enzyme activity from eight experiments for euthyroid and three experiments for hypothyroid cells. Steady-state enzyme levels were 84 ± 9 (S.D.) units/mg protein and 1230 ± 63 (S.D.) units/mg protein in the presence or absence of serum, respectively.

**TABLE IV**
Effects of cytoskeletal, lysosomotropic, and endocytosis inhibitors and carbonyl cyanide m-chlorophenylhydrazone (CCCP) on the turnover of type II 5'-deiodinase in bt,cAMP-stimulated glial cells

Triplicate flasks (25 cm2) of glial cells grown in euthyroid medium were stimulated for 16 h with 1 mM bt,cAMP. At the start of the experimental period, test compounds were added in the absence or presence of 500 μM cycloheximide and cells incubated for the times indicated. Cells were then harvested by scraping and 5'-deiodinating activity determined as described under "Materials and Methods." Data are expressed as % of untreated control enzyme activity from closely agreeing (±10%) triplicates.

| Inhibitor | Time | Type II 5'-deiodinase activity |
|-----------|------|-----------------------------|
| Control   | min  | % control activity          |
| 0.5 mM cycloheximide |       |                             |
| No addition | 30     | 100                          | 22 |
| + Dihydrocytochalasin B (10 μM) | 60     | 202                          | 60 |
| + Cytochalasin B (15 μM) | 30    | 139                          | 71 |
| + Colchicine (20 μM) | 60     | 243                          |     |
| + Chloroquine (0.2 mM) | 30     | 125                          | 19 |
| + NH₄Cl (10 mM) | 30     | 78                           | 29 |
| + Tunicamycin (0.25 μM) | 30  | 90                           | 32 |
| + Monodansylcadaverine (0.1 mM) | 30   | 71                           | 7  |

**Fig. 6. Effects of cytochalasin B on the disappearance of type II 5'-deiodinase (5',D-II) in cycloheximide-blocked cells.**

Steady-state levels of type II 5'-deiodinase were induced as described under "Materials and Methods" in cells grown in DMEM containing 10% fetal bovine serum. At the start of the experiment, cycloheximide (100 μM final concentration) in the absence or presence of 15 μM cytochalasin B was added to triplicate flasks (25 cm2) and the cultures incubated at 37 °C for the times indicated. Enzyme was determined in cell sonicates as described under "Materials and Methods." Data are reported as % of remaining activity. The numbers in parentheses indicates the number of individual experiments performed, each done in triplicate.
that found in rat brain (5–8, 29). Comparison of the enzyme production rates in cells grown in euthyroid and hypothyroid media revealed that thyroid hormone had no effect on synthesis; the increased 5'-deiodinating activity observed in hypothyroid cultures was entirely accounted for by diminished enzyme degradation/inactivation. These data are in agreement with earlier work that showed that the $t_\text{1/2}$ for cerebrocortical type II 5'-deiodinase was prolonged 10–15-fold in the hypothyroid rat (8) and further that the acute effects of thyroid hormone on the enzyme $t_\text{1/2}$ did not require continued transcription or translation (8). Thus, cAMP-stimulated gial cell faithfully mimics the intact rat brain with respect to thyroid hormone-dependent regulation of this key membrane-bound enzyme.

The influence of individual iodothyronines on steady-state enzyme levels in the stimulated astrocyte was identical to those previously determined in vivo. T$_4$ and T$_3$, a metabolically inactive iodothyronine, were ~100-fold more potent than T$_3$ in modulating enzyme levels, as had been reported by Silva and Leonard (7) and Kaiser et al. (10) in vivo, and by St. Germain (29) in NB41A3 cells, and half-maximal hormonal effects were observed with biologically relevant concentrations. The rank order of potency of this limited series of iodothyronines differs markedly from that for nuclear T$_3$ receptor (for reviews see Refs. 30, 31) and is consistent with the proposed extra-nuclear site of action.

Additional support for an extranuclear site of thyroid hormone action is provided by the observation that under our culture conditions few, if any, nuclear T$_3$ receptors are present in glial cells (32). In contrast, others have reported modest levels of nuclear T$_3$ receptors in cultured glial cells obtained from primary dispersions of fetal mouse cerebral cortex grown without further subculture (33) and in C-6 astrocytomas cells (34, 35). Thus, it seems likely that continued subculture of astrocytes may result in the loss of the nuclear T$_3$ receptor, without affecting the ability of the cell to respond to thyroid hormone by modulating type II 5'-deiodinase.

Our preliminary survey of potential cellular mechanisms mediating the T$_3$-dependent modulation of the degradation/inactivation of the membrane-bound enzyme revealed that this was an energy-dependent process requiring an intact actin-cytoskeleton. Lysosomotropic agents and inhibitors of endocytosis and glycosidation had little or no effect on type II 5'-deiodinase suggesting that neither enzyme internalization by selective endocytosis nor glycosidation during synthesis contributed to the short half-life of the enzyme. The failure of colchicine to influence the dynamics of type II 5'-deiodinase induction and/or inactivation, despite a profound effect on the morphology of the bg,cAMP-stimulated astrocyte indicates that turnover of this enzyme does not require intact microtubules.

Both adequate intracellular ATP and an intact actin-cytoskeleton were found to be essential for the rapid inactivation/degradation of membrane-bound type II 5'-deiodinase. Carboxyl cyanide m-chlorophenylhydrazone-treated cells showed the expected modest decrease in protein synthesis and therefore type II 5'-deiodinase synthesis. However, enzyme activity was not further diminished by complete inhibition of protein synthesis with cycloheximide. These data demonstrate that in addition to the well-known energy dependence of translation, inactivation/degradation of type II 5'-deiodinase requires ATP.

The most impressive antagonists of type II 5'-deiodinase turnover were cytochalasins. Cytochalasins halted the loss of type II 5'-deiodinase in cells unable to synthesize protein and stimulated further accumulation of enzyme in cells capable of translation. Since cytochalasin B is an affinity ligand for the glucose transporter (36, 37), as well as an inhibitor of microfilament polymerization (38), we used the microfilament-specific derivative, dihydrocytochalasin B (39), and both compounds yielded identical results. In preliminary studies, short-term glucose depletion (4 h) had no effect on the disappearance kinetics of 5'-deiodinating activity in astrocytes grown in euthyroid medium, demonstrating that the cytochalasin effect was mediated through its ability to depolymerize the actin-cytoskeleton, rather than by altering glucose entry.2

The specific interrelationships between the actin-cytoskeleton and the rapid inactivation/degradation of type II 5'-deiodinase remain to be determined. It is clear, however, that dynamic regulation of the structural integrity of the actin-cytoskeleton may play an important role in the inactivation pathway for this membrane-bound enzyme and thereby exert a direct effect on steady-state levels of the enzyme catalyzing intracellular T$_3$ production in the brain. Recent studies by St. Germain (9) have attempted to address this issue by examining the nature of the thyroid hormone dependence of type II 5'-deiodinase turnover in neuroblastoma cells. A parallelism between the substrate specificity and enzyme cofactor requirements and the ability of these compounds to alter enzyme inactivation in NB41A3 cells led to the proposal that substrate-induced inactivation of type II 5'-deiodinase is a key factor in determining the biological half-life, and thereby cellular levels, of this enzyme. Our demonstration that disruption of the actin-cytoskeleton abolishes the inactivation of type II 5'-deiodinase in astrocytes grown in the presence of thyroid hormone suggests that substrate-enzyme interactions alone are insufficient to account for the T$_3$-dependent modulation of type II 5'-deiodinase turnover.

Thus, in a cell culture model system mimicking all of the known regulatory pathways of type II 5'-deiodinase in the brain, the T$_3$-dependent regulation of biological half-life of the enzyme is mediated by an energy-dependent process requiring an intact actin-cytoskeleton. Characterization of the interactions between thyroid hormone and the actin-cytoskeleton, without involvement of the nuclear T$_3$ receptor, should provide new insights into the potential mechanisms available for this metabolically potent hormone to modulate neuronal arborization, cell-cell communication, and the structural abnormalities observed in the brain in congenital hypothyroidism.

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