Dual Mechanisms of Regulation of Type I Iodothyronine 5'-Deiodinase in the Rat Kidney, Liver, and Thyroid Gland

Implications for the Treatment of Hyperthyroidism with Radiographic Contrast Agents

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Abstract

Alterations in thyroid hormone status and the administration of radiographic contrast agents can markedly influence iodothyronine metabolism and, in particular, the activity of type I 5'-deiodinase (5'DI). In the present studies, the mechanisms responsible for these effects have been reassessed. As previously reported, the addition of iopanoic acid (IOP) to broken cell preparations resulted in a competitive pattern of 5'DI inhibition. However, the in vivo administration to rats of IOP or 3,3',5'-triiodothyronine (rT3) resulted in a noncompetitive pattern of inhibition of 5'DI in the liver, kidney, and thyroid gland, whereby marked decreases in maximal enzyme velocity (V_{max}) were noted, with no change in the value of the Michaelis-Menten constant. In rats rendered hyperthyroid by the injection of 3,5,3'-triiodothyronine (T3), 5'DI activity was significantly increased in the liver and the kidney. The administration of IOP to these thyrotoxic animals resulted in a rapid loss of enzyme activity characterized by an approximate 80% decrease in 5'DI V_{max} values in both tissues. Furthermore, this inhibitory effect persisted for longer than 60 h after a single IOP injection. IOP administration also decreased 5'DI V_{max} levels in the thyroid gland by 52%. In other experiments, treatment of intact Reuber FAO hepatoma cells with IOP or rT3 induced a rapid decrease in 5'DI V_{max} levels. In cells treated with cycloheximide, these agents enhanced the rate of disappearance of enzyme activity by > 12-fold, indicating a predominant effect on accelerating the rate of enzyme inactivation and/or degradation. These studies demonstrate that iodothyronines and other iodinated compounds have complex regulatory effects on 5'DI that entail alterations in the rates of both enzyme activation and inactivation. The previously accepted concept that rT3 and IOP impair thyroxine (T4) to T3 conversion in vivo by acting as competitive inhibitors is an oversimplification. Rather, the clinically beneficial effects of administering these agents to patients with hyperthyroidism may result primarily from the rapid and prolonged inactivation of 5'DI which occurs in the thyroid gland and peripheral tissues.

Introduction

The hyperthyroid state associated with Graves' disease is characterized by a marked increase in the rate of both the thyroidal secretion and the peripheral production of 3,3',5'-triiodothyronine (T3) (1). The resultant elevations in the circulating and tissue T3 concentrations lead to functional changes in a number of organ systems and thus produce the characteristic signs and symptoms of this disorder.

Alterations in thyroid hormone status also have important and direct "autoregulatory" effects on the cellular processes that metabolize these hormones (2). In the rat, hyperthyroidism results in a marked increase in the rate of "local" T3 production in the liver (3, 4). This effect is secondary, in part, to an increase in activity of type I 5'-deiodinase (5'DI), the principal enzymatic process responsible for hepatic thyroxine (T4) to T3 conversion (5). In contrast to this activating effect of thyroid hormones on 5'DI in the liver (and kidney [6]), type II 5'-deiodinase (5'DII) activity in the anterior pituitary gland and central nervous system is suppressed in hyperthyroid animals, and T3 neogenesis in these tissues is markedly decreased (2, 3). The autoregulatory effects of thyroid hormones on 5'DI and 5'DII thus differ significantly. As a consequence, the 5'DI present in the liver and kidney is likely to be the principal process mediating the extrathyroidal conversion of T4 to T3 in the hyperthyroid state.

Radiographic contrast agents such as iopanoic acid (IOP) and sodium ipodate (NaIp) inhibit T4 to T3 conversion in man (7-9) and experimental animals (10-12), and offer an alternative to the thionamides in the treatment of Graves' disease (13, 14). When administered to hyperthyroid patients, these agents induce a marked decrease in the serum T3 concentration which occurs more rapidly than that noted after the administration of stable iodine (15) or 6-n-propyl-2-thiouracil (PTU) (16). The rapidity with which radiographic contrast agents lower T3 levels may be of considerable clinical importance. In a recent study of thyrotoxic patients with severe cardiac manifestations, the amelioration of clinical symptoms after NaIp administration paralleled the fall in serum T3 levels; significant improvements in several cardiovascular parameters were noted as early as 3 h after a single dose (17).

In tissue homogenates, IOP and NaIp act as competitive inhibitors of both 5'DI (18-20) and 5'DII (21, 22), and this has previously been assumed to be the mechanism whereby they

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1. Abbreviations used in this paper: BW, body weight; 5'DI, type I iodothyronine 5'-deiodinase; 5'DII, type II iodothyronine 5'-deiodinase; IOP, iopanoic acid; MMI, methimazole; NaIp, sodium ipodate; prot, protein; PTU, 6-n-propyl-2-thiouracil; rT3, 3,3',5'-triiodothyronine; T3, 3,3',5'-triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone.
inhibit T₃ neogenesis in vivo. We have recently demonstrated, however, that these agents inhibit T₄ to T₃ conversion in the anterior pituitary gland and cerebral cortex in vivo by non-competitively and irreversibly inactivating the 5'DII enzyme (23). This process of inactivation involves a unique mechanism of enzyme regulation that may be initiated by the direct interaction of ligands (e.g., substrates such as T₄ or 3,3',5'-triiodothyronine [reverse T₃, rT₃]), or competitive inhibitors such as IOP) with the 5'DII active site. Furthermore, this ligand-induced inactivation of 5'DII is modulated by the cellular thiol/disulfide balance (23).

These findings regarding the regulation of 5'DII prompted us to investigate the mechanisms whereby thyroid hormones, radiographic contrast agents, and sulphydryl oxidizing agents regulate 5'DI in several tissues of the rat. Our results demonstrate that 5'DI is regulated in a manner considerably more complex than previously believed, and that this enzymatic process in the liver, kidney, and thyroid gland is subject to ligand-induced inactivation in a fashion analogous to that previously delineated for 5'DII.

Methods

Materials. Iodothyronines were obtained from Henning Co. (West Berlin, FRG) or Calbiochem-Behring Diagnostics, American Hoechst Corp. (La Jolla, CA) and were of the highest purity commercially available. IOP was kindly provided by the Sterling-Winthrop Research Institute (Rensselaer, NY). 3'- or 5',25I-labeled rT₃ (SA 380 mCi/µg) was obtained from New England Nuclear (Boston, MA).

Animal experiments. Male Sprague-Dawley rats (175–200 g) were used in all experiments and were obtained from Charles River Breeding Laboratory, Inc. (Wilmington, MA). Animals were housed under conditions of controlled lighting and temperature and were given free access to food. In one experiment, rats were rendered hypothyroid and goitrous by the inclusion of 200 µg/ml methimazole (MMI) in their drinking water for 4 wk. Animals so treated stopped gaining weight after 10 d, and the wet weight of their individual thyroid glands was increased threefold over that of euthyroid control animals (control, 14±1 mg/gland; MMI, 41±2; P < 0.001). In other experiments, thyroidecatedomized rats (with the parathyroid glands reimplanted) were obtained from the same supplier and were used for experiments 8 wk after surgery. Before killing, animals received single or multiple injections of T₁, rT₃, T₄, and/or IOP according to the experimental protocols described below. Control animals received equivalent volumes of the vehicle solution (propylene glycol, 0.1 N NaOH, 0.25 M NaCl [50:42:8 vol/vol]).

Renal, hepatic, and/or thyroidal tissue were harvested from individual animals and homogenized in 10 vol of assay buffer (0.25 M sucrose, 0.02 M Tris/HCl, pH 7.0, 1 mM EDTA) using either a motorized Teflon pestle (kidney and liver) or a ground glass homogenizer (thyroid). The homogenates were centrifuged at 1,000 g and the supernatant diluted 1:40 to 1:800 with assay buffer. All preparative procedures were performed at 0–4°C. 5'DI activity was determined immediately in the diluted supernatant by a kinetic analysis in which the production rate of 123I⁻ from 123I-rT₃ was measured in the presence of 20 µM diithiothreitol using a modification of the methods of Leonard and Rosenblatt (24) and McCann et al. (25). At each concentration of 123I-rT₃, duplicate aliquots of the diluted supernatant (40 µl containing 0.5–10 µg of protein) were incubated at 37°C for 40 min and the reaction was then terminated by the addition of 12 µl of an ice-cold BSA solution (40 mg/ml) plus 120 µl of an ice-cold 2% TCA solution. Other aliquots were kept at 0–4°C and terminated immediately after adding the 123I-rT₃ to determine basal 123I⁻ content. The 123I⁻ present in the supernatant fraction of the terminated reaction mixture was separated by ion-exchange chromatography using an AG50W-X8 (H⁺) column (mesh size, 200–400; bed size, 0.7 × 4 cm; Bio-Rad Laboratories, Richmond, CA) equilibrated and washed with 10% acetic acid. The 123I⁻ in the column effluent was counted in a gamma counter. As quantified by paper chromatography or TLC (see below), equal molar quantities of 3,3',5'-triiodothyronine and 1⁻ were produced from 123I-rT₃ in the homogenates of kidney, liver, and thyroid tissue. No 123I⁻ was released in tissue-free incubations performed at 37°C. 5'DI activity was expressed as picomoles of 123I⁻ released per minute per milligram protein after multiplying by a factor of two to correct for the random labeling of 123I-rT₃ at the 3' or 5' position. Kinetic data were analyzed by double reciprocal and Eadie-Hofstee plots (26) using the average substrate concentration during the 40-min incubation period as suggested by Lee and Wilson (27). The value of the Kᵢ was calculated by reploting the slope of the double reciprocal plot vs. the concentration of competitive inhibitor. On such a plot, the Kᵢ value is represented by the negative value of the x-intercept (28).

Cell culture experiments. Reuber FAO rat hepatoma cells were a gift from Dr. Lee A. Witters (Dartmouth Medical School, Hanover, NH) and were routinely grown in 75-cm² plastic flasks and 60- and 100-mm plastic culture dishes using RPMI 1640 culture medium supplemented with 5% FCS, 5% calf serum, 100 U/ml penicillin, and 100 µg/ml streptomycin. In some studies, cells were transfected and maintained for 24–48 h in serum-free RPMI 1640 medium before experiments. Other experiments were performed with cells incubated in serum-free medium which was also glucose-free. Experiments were performed with cells at the confluent stage of growth and 24 h after the last medium change. At various times after the addition of agents to the FAO cell cultures, the medium was aspirated and the cells were washed twice with PBS. The cells were then harvested by scraping with a rubber policeman into assay buffer and sonicated using a sonic dismembrator (Artrek Systems Corp., Dynatech Corp., Farmingdale, NY). In experiments in which cells were incubated with diamide, the wash buffer and assay buffer contained 5 mM DTT. The sonicate was then centrifuged at 14,000 g and aliquots of the supernatant (40 µl containing ∼25–100 µg of protein) were used immediately for the determination of 5'DI activity as described above. All preparative procedures were carried out at 0–4°C. As quantified by paper chromatography, equal molar quantities of 3,3'-T₃ and 1⁻ were produced from 123I-rT₃ in the FAO cell sonicates.

Other determinations. The effect of cycloheximide on protein synthesis in FAO cells was determined by quantitating [3H]leucine incorporation into TCA-precipitated material according to the method of Scornik et al. (29).

As cementing paper chromatography was performed using a tertial amyl alcohol/2 N ammonium hydroxide (1:1) solvent system as described by Galton and Hiebert (30).

TLC was performed as described by van Doorn et al. (31) using 20 × 20 cm aluminum sheets coated with Silicagel-60 (E. Merck, Darmstadt, FRG) and a solvent system consisting of 25% ammonium-methanol-chloroform (3:2:0:40 vol/vol). RI values for the iodinated compounds of interest were: 0.31 for rT₃, 0.42 for 3,3'-T₃, and 0.67 for 1⁻.

Protein concentrations were determined by the method of Lowry et al. (32). Aliquots of tissue samples containing DTT were precipitated with TCA before the determination of protein content (33).

All results are given as the mean ±SE. Statistical analysis was performed using the paired and unpaired t test with the Bonferroni correction applied when multiple comparisons were made (34). Alternatively, Dunnett's t test was used when multiple comparisons were made with a control group (35).

Results

Regulation of 5'DI in kidney and liver. As previously reported by other investigators (18–20), IOP acted as a competitive inhibitor of 5'DI in vitro. Thus, the addition of increasing concentrations of IOP to kidney homogenates from a euthyroid animal resulted in a series of kinetic curves that inter-
sected at the y-axis on a double reciprocal plot (Fig. 1). Slope-
replots of such data (Fig. 1, inset) from two experiments
yielded $K_v$ values of 8 and 10 μM.

The in vivo administration of IOP to thyroidectomized rats
resulted in an entirely different pattern of inhibition. Double
reciprocal plots of kinetic data obtained in renal homogenates
from individual animals injected 3 h previously with IOP re-
vealed a decrease in $V_{max}$ with no change in the value of the
$K_v$, indicating a noncompetitive mechanism of inhibition
(Fig. 2). As shown in Table I, IOP dosages of 0.04 and 4
mg/100 g body weight (BW) inhibited 5'DI $V_{max}$ values by 52
and 66%, respectively. The finding that $K_v$ values were un-
changed in this (Table I), as well as in subsequent experiments,
indicates that the amount of IOP carried over into the in vitro
5'DI assay system was insufficient to cause competitive inhibi-
tion of 125I-T3 deiodination.

The effects of the chronic administration of T3, rT3, and
IOP on the regulation of 5'DI in the kidney and liver were ex-
amined in a second group of thyroidectomized rats (Fig. 3).
Twice daily, injections of rT3 (50 μg/100 g BW) or IOP (100
μg/100 g BW) resulted in a marked and significant decrease in
$V_{max}$ values in both tissues. In contrast, the administration of
T3 in a dose designed to render the animals hyperthyroid (0.6
μg/100 g BW, twice daily) increased 5'DI activity to levels
10-fold greater than those observed in hypothyroid animals
and two- to threefold greater than those present in a control
group of euthyroid rats of the same age. The ability of rT3 and
IOP to antagonize the stimulating effects of T3 were also ex-
amined (Fig. 3). 5'DI activity in hyperthyroid animals was un-
changed after a single injection of rT3 (50 μg/100 g BW) or IOP
(100 μg/100 g BW). However, injection of a single larger dose
of IOP (4 mg/100 g BW), comparable in amount to that used
in treating hyperthyroid patients with Graves' disease, resulted
in a 78 and 85% decrease in $V_{max}$ values in the liver and kidney,
respectively. This inhibition by IOP in thyrotoxic animals re-
resulted in levels of 5'DI activity that were less than or similar to
those noted in euthyroid control rats. Again, $K_v$ values in all
groups were unchanged (Fig. 3), which suggests that the ob-
erved alterations in enzyme activity represented changes in
the cellular content of active enzyme molecules. Kinetic data
obtained in the liver from representative, individual animals
from several of the treatment groups are shown in Fig. 4. On
such plots, the $V_{max}$ value is represented by the y-intercept,
whereas the $K_v$ value is equal to the negative value of the
slope.

The time course of the in vivo inhibitory effects of IOP on
5'DI in hyperthyroid animals was investigated in another ex-
periment in which rats were treated chronically with T3 (1
μg/100 g BW, s.c., twice daily) and then were administered a
single intravenous dose of IOP (4 mg/100 g BW) at 5, 12, 24,
or 60 h before killing (Fig. 5). In the 24- and 60-h treatment
groups, T3 administration was continued until 12 h before
killing. Hyperthyroid animals again demonstrated signifi-
cantly higher 5'DI activity in the liver and kidney when com-
pared with euthyroid control rats. IOP administration resulted
in an inhibition of $V_{max}$ values in both tissues which was rapid
in onset and prolonged in duration. The maximal inhibitory
effects of 77 and 84% in liver and kidney, respectively, were
similar to those noted previously and occurred at 5–12 h after

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Table I. Effects of the In Vivo Administration of IOP on 5'DI Activity in the Kidney of Hypothyroid Rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$n^*$</th>
<th>$V_{max}$ (pmol/min×mg protein)</th>
<th>$K_v$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>13.2±0.7</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>IOP (0.04)</td>
<td>5</td>
<td>6.4±0.6&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>IOP (4)</td>
<td>5</td>
<td>4.5±0.3&lt;sup&gt;5&lt;/sup&gt;</td>
<td>0.11±0.03</td>
</tr>
</tbody>
</table>

* $n$, number of rats.

<sup>4</sup> IOP, in a single dose of 0.04 or 4 mg/100 g BW, i.p., was adminis-
tered 3 h before killing to rats that had been thyroidectomized 8 wk
earlier. Data represent the mean±SE.

<sup>5</sup> $P < 0.001$ vs. control (Dunnett's t test).
**Figure 3.** Effects of the chronic and acute in vivo administration of rT3, IOP, and T3 on 5'IDI activity in the (A) liver and (B) kidney. Thyroidectomized rats received twice daily subcutaneous injections for 4 d according to the following protocol: (a) control, vehicle solution; (b) rT3, 50 μg/100 g BW; (c) IOP, 100 μg/100 g BW; (d) T3 treatment, 0.6 μg/100 g BW. Animals were killed 12 h after the last injection and 5'IDI activity was quantitated in diluted supernatant fractions of liver and kidney homogenates. 6 h before killing, T3-treated animals received a single intravenous injection of vehicle (veh), rT3 (50 μg/100 g BW), or IOP (100 μg or 4 mg/100 g BW). A group of euthyroid control rats of the same age served as an additional control group. Results depict the mean±SE of four to five animals per group. The insets at the base of each bar report the mean Km value of each group expressed in μmol/liter. *P < 0.01 vs. thyroidectomized control group; †P < 0.01 vs. T3 vehicle treatment group; ‡P < 0.05 vs. thyroidectomized control; §P < 0.01 vs. T3 vehicle treatment group and vs. euthyroid control group.

The IOP injection. Although partial recovery of 5'IDI activity was noted at the 60-h time point, Vmax values were still significantly less (36 and 18% in liver and kidney, respectively) than those noted in the corresponding hyperthyroid control group.

**Effects of IOP on 5'IDI activity in the thyroid gland.** 5'IDI activity has been reported to be increased in the thyroid gland of patients with Graves' disease (36, 37). Similar increases in activity have been noted in the thyroid glands of rodents that were administered thyroid-stimulating hormone (TSH) (38) or Igs from patients with Graves' disease (39), or rendered hypothyroid by the chronic administration of MMI (40). To determine whether radiographic contrast agents inhibit 5'IDI Vmax levels in thyroid tissue, rats made goitrous by the inclusion of MMI in their drinking water were injected intravenously with a single dose of IOP (4 mg/100 g BW) and then killed 5 or 12 h later. In contrast to a previous report (40), 5'IDI activity in the thyroid glands of MMI-treated rats was not different from that determined in euthyroid control rats of the same age (Table II). IOP administration, however, resulted in a significant decrease in Vmax levels at both time points. At 5 h after the IOP injection, 5'IDI activity was decreased by 29% (P < 0.05), whereas at the 12-h time point activity was reduced by 52% (P < 0.005). IOP administration did not alter Km values in either treatment group. Thus, as is the case in the liver and the kidney, radiographic contrast agents inhibit 5'IDI in the thyroid gland in vivo by a noncompetitive mechanism.

**Regulation of 5'IDI in the Reuber FAO hepatoma cell line.** The suitability of Reuber FAO hepatoma cells to serve as a model system for studying the regulation of 5'IDI activity was investigated. Phenolic ring deiodinase activity was easily demonstrated in FAO cell sonicates using 125I-rT3 as substrate. Characterization of this deiodinating process revealed the following: (a) 125I release from 125I-rT3 was thiol-dependent with maximal product formation noted at DTT concentrations of 5–20 mM; (b) no 125I production was noted in cell sonicates incubated at 0–4°C or in sonicates previously heated to 80°C for 30 min; (c) 125I- production varied linearly with sonicate protein content up to 2.5 mg/ml; (d) as determined by paper chromatography, equal quantities of I⁻ and 3,3'-diiodothyronine were formed from 125I-rT3; (e) in the presence of 20 mM dithiothreitol, PTU at a concentration of 1 mM inhibited 125I- production by >99%; (f) using 1 nM 125I-rT3 as a substrate, no phenolic or tyrosyl ring deiodinating activity could be demonstrated in cell sonicates under routine assay conditions; (g) 125I- formation decreased somewhat with time of assay incubation; calculated deiodinase activity at the end of a 40-min incubation period was decreased by ~30% from that determined during a 10-min incubation. However, to allow accurate quantitation of reaction velocity under a variety of experimental conditions, a 40-min incubation period was typically used. Using 125I-rT3 as substrate in sonicates prepared from FAO cells that were maintained in serum-free medium, saturable reaction kinetics were demonstrated for phenolic ring
deiodinase activity. In seven experiments, mean $K_m$ and $V_{\text{max}}$ values were 0.22±0.02 μM and 5.3±1.1 pmol 125I- formed/min · mg protein, respectively. Reuber FAO hepatoma cells thus manifest a 5'deiodinase process that has the typical characteristics of the type I activity normally found in rat liver and kidney.

The addition of IOP or rT3 to the culture medium of FAO cells maintained under serum-free conditions resulted in a dose- and time-dependent inhibition of 5'DI activity that was characterized by a decrease in $V_{\text{max}}$ with no change in the $K_m$ value (Fig. 6). In three experiments in which cells were incubated for 18–40 h with 0.5 μM IOP, $V_{\text{max}}$ values were decreased by an average of 51±5%. At higher IOP concentrations (10 μM), inhibition was as great as 88%. The inhibitory effects of rT3 were approximately equipotent to those of IOP. $V_{\text{max}}$ values were decreased by 36±6, 46±8, and 90±1% in three experiments in which cells were incubated with rT3 at concentrations of 0.1, 1, and 3.3 μM, respectively. In other studies, a similar time course of 5'DI inhibition was noted for IOP and rT3; inhibitory effects were half-maximal at 3 h and maximal at 20 h after addition of the agents to the culture medium.

The dependency of the inhibitory effects of IOP and rT3 on protein synthesis was investigated using FAO cells incubated with cycloheximide (Fig. 7). The addition of 50 μg/ml cycloheximide to the culture medium inhibited [3H]leucine incorporation into TCA-precipitable material by > 95%. In spite of this inhibition of protein synthesis, cycloheximide treatment for 6 h decreased 5'DI activity in FAO cells by only 5±2% (n = 4 experiments) compared with untreated control cells. Cycloheximide did not block the inhibitory effects of IOP and rT3 on 5'DI activity. The addition of rT3 (1 μM) or IOP (10 μM) to cycloheximide-treated cells resulted in a 42±9 and 53±10% decrease ($P < 0.01$ for either group vs. cycloheximide treatment alone) in enzyme activity, respectively, after a 6-h incubation. Thus, inhibition of 5'DI by these agents is due to an enhanced rate of enzyme inactivation and/or degradation.

A striking feature of the regulation of phenolic ring deiodination in GH3 pituitary tumor cells is that exposure of intact

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$n$*</th>
<th>$V_{\text{max}}$ (pmol/min · mg protein)</th>
<th>$K_m$ (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Euthyroid</td>
<td>7</td>
<td>163±13</td>
<td>0.09±0.01</td>
</tr>
<tr>
<td>MMI control†</td>
<td>6</td>
<td>137±10</td>
<td>0.12±0.01</td>
</tr>
<tr>
<td>MMI + IOP (5 h)</td>
<td>5</td>
<td>97±12</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>MMI + IOP (12 h)</td>
<td>5</td>
<td>66±7</td>
<td>0.12±0.02</td>
</tr>
</tbody>
</table>

* $n$, number of rats.
† Rats were rendered hypothyroid and goitrous by the inclusion of 200 μg/ml MMI in their drinking water for 4 wk. IOP, in a single dose of 4 mg/100 g BW, was administered intravenously 5 or 12 h before killing. MMI control animals were injected with an equivalent volume of vehicle. Rats maintained for the same period on regular drinking water served as a euthyroid group. Data represent the mean±SE.
‡ $P < 0.05$ vs. MMI control.
§ $P < 0.005$ vs. MMI control. Statistical analysis was performed with Dunnnett’s $t$ test.

![Figure 5](image_url)

*Figure 5.* Time course of the inhibitory effect of IOP on 5'DI activity in the (A) liver and (B) kidney of hyperthyroid rats. Euthyroid rats were rendered hyperthyroid by the subcutaneous injection of T3 (1 μg/100 g BW twice daily) for 4 d. The hyperthyroid rats were then administered a single, intravenous dose of vehicle solution or IOP (4 mg/100 g BW) and killed 5, 12, 24, or 60 h later. T3 injections were continued until 12 h before killing. Animals receiving a subcutaneous injection of vehicle twice daily for 4 d served as a euthyroid control group. Results depict the mean±SE of four to five animals per group. The insets at the base of each bar report the mean $K_m$ value of each group expressed in μmol/liter. *$P < 0.01$ vs. corresponding hyperthyroid, vehicle group; †$P < 0.05$ vs. corresponding hyperthyroid, vehicle group.

![Figure 6](image_url)

*Figure 6.* Effects of IOP and rT3 on 5'DI activity in intact, cultured Reuber FAO hepatoma cells. FAO cells were grown to confluence in serum-containing medium, transferred, and maintained for 24 h in serum-free RPMI 1640 medium. IOP or rT3, in the final concentrations shown, were then added to the medium and the cells were cultured for an additional 20 h. Cells were then harvested and 5'DI activity was determined in supernatant fractions of cell sonicates. The data depicted are Eadie-Hofstee plots from a single, representative experiment. A noncompetitive pattern of inhibition is demonstrated. Concentrations of rT3 on the abscissa are expressed in μmol/liter.
Figure 7. The effects of rT₃ and IOP on 5'DI activity in intact, cultured FAO cells treated with cycloheximide. FAO cells were grown to confluency in serum-containing medium and then transferred and maintained for 24 h in serum-free RPMI 1640 medium. Cycloheximide (50 μg/ml), with or without rT₃ (1 μM) or IOP (10 μM), was then added to the medium and the cells were cultured for an additional 6 h. Cells were then harvested, and 5'DI activity was determined in supernatant fractions of cell sonicates. FAO cells maintained in serum-free medium alone served as controls. The data depicted represent the mean±SE of the pooled results from four experiments. *P < 0.01 vs. the cycloheximide group by Dunnett's t test.

cells to the sulphydryl oxidizing agent diamide results in a rapid inactivation of 5'DI activity which mimicks the effects of rT₃, IOP, and other ligands (23). Analogous findings were demonstrated in FAO hepatoma cells treated with diamide (Fig. 8). In three experiments where intact cells were incubated for 3.5 h with 0.4 mM diamide, 5'DI Vₘₐₓ levels decreased by 75±4% (P < 0.025), whereas Kₘ values were unaltered. Given the prior observation that the inhibition of protein synthesis for 6 h in FAO cells resulted in only a minimal loss of 5'DI activity, the rapidity of the diamide effect necessitates that the mechanism involved is an enhanced rate of enzyme inactivation. In these experiments, cells were maintained during the 3.5-h incubation period in glucose-free medium to inhibit the reformation of glutathione (41). 5'DI activity in control cells maintained in this medium did not differ from that noted in cells maintained concurrently in the same medium supplemented with glucose (2 mg/ml). The inhibitory effect of diamide in these experiments was not due to interference in the in vitro 5'DI assay system; the addition of 1 mM diamide to FAO cell sonicates did not alter 5'DI activity when 20 mM DTT was present in the reaction mixture.

Discussion

The two enzymatic pathways that convert T₄ to T₃ (i.e., 5'DI and 5'DII) have been previously distinguished by differences in kinetic characteristics, susceptibility to inhibition by PTU, tissue distribution, and response to alterations in thyroid hormone status (42). Whereas hyperthyroidism in the rat leads to increased 5'DII activity (5, 6), the activity of 5'DI is markedly suppressed due to an unusual, and as yet incompletely understood process, which we have termed ligand-induced inactivation (23). The seminal finding of the present studies is that substrates and competitive inhibitors also induce the inactivation of 5'DI in vivo. This finding has significant implications for our understanding of the cellular mechanisms that regulate thyroid hormone economy.

Inactivation of phenolic ring deiodinases. In the experiments reported herein, the administration of iodinated compounds that are devoid of thyromimetic activity (i.e., rT₃ or IOP) to experimental animals or cultured cells resulted in a rapid and prolonged suppression of 5'DI activity that was characterized by a decrease in Vₘₐₓ with no change in Kₘ. These effects on 5'DI in hepatic and renal tissue are exactly analogous to those previously defined for 5'DII in the anterior pituitary gland and cerebral cortex (23). Furthermore, the inhibitory effects of ligands on these processes are independent of protein synthesis and are, therefore, secondary to an enhanced rate of enzyme inactivation and/or degradation. In the case of the 5'DII present in FAO cells, rT₃ and IOP enhanced the rate of loss of enzyme activity by greater than 12-fold, assuming that the disappearance of activity after cycloheximide treatment is exponential.

Sato et al. (41) have previously reported that diamide treatment impairs the conversion of T₄ to T₃ by intact, cultured rat hepatocytes. Their studies, however, did not determine whether the decrease in T₃ formation was due to a decrease in the availability of a necessary thiol cofactor or if it was secondary to an effect on 5'DI synthesis or inactivation. The present experiments demonstrate that this inhibitory effect of diamide on iodothyronine metabolism is due to a rapid inactivation of 5'DI. We have previously demonstrated that ligand-induced inactivation of 5'DII is also influenced by the cellular thiol/disulfide balance; the exposure of intact GH₃ cells to sulphydryl oxidizing agents rapidly inactivates the enzyme, whereas exposure to sulphydryl reducing agents protects against the substrate-induced loss of activity (23). Thus, the present finding that diamide rapidly inactivates 5'DI in intact FAO cells provides another important parallel in the regulation of the two phenolic ring deiodinase processes.

Of note, however, is that the 5'DII process appears to be considerably less sensitive than the 5'DI one to ligand-induced inactivation. Under experimental conditions where enzyme activity is increased (hypothyroidism for 5'DII; hyperthyroidism for 5'DI), a single injection of 0.04 mg IOP/100 g BW inactivates > 80% of 5'DII in the anterior pituitary gland and cerebral cortex (23), yet has no effect on 5'DI in the liver or 1481.
kidney (Fig. 3). Indeed, a 70–80% inhibition of 5DI activity is only achieved when hypothyroid or hyperthyroid rats are administered a 100-fold larger dose of IOP (Table 1 and Fig. 3). Similarly, a single dose of rT3 of 50 μg/100 g BW, which has been demonstrated by other investigators to inhibit 5DII activity in the pituitary gland and cerebral cortex by > 90% (43), had no effect on 5DI activity when administered to hyperthyroid animals in the present studies. Results from our cell culture studies provide additional evidence that 5DI is less sensitive to ligand-induced inactivation. We have previously demonstrated that the concentration of IOP required to inhibit 5DII activity by 50% in intact GH3 cells that are maintained in serum-free medium is 0.005 μM (23). In the present experiments using FAO cells grown under analogous conditions, 50% inhibition of 5DI required a medium IOP concentration of 0.5 μM.

Taken together, these data suggest that ~100-fold greater concentrations of ligand are required to inactivate 5DI as compared with 5DII. The reason for this marked difference in susceptibility to inactivation is uncertain, but could reflect tissue differences in either ligand concentration or the thiol/disulfide balance. Another intriguing possibility is suggested by our previous observation that the potency of a ligand in inactivating 5DII in intact GH3 cells is closely correlated with the ligand’s Ki value as determined in broken cell preparations and indicative of the affinity of the ligand for the enzyme’s active site (23). The Ki values of IOP for 5DI and 5DII are 8 μM (Fig. 1) and 0.05 μM (23), respectively, and therefore the affinity of 5DI for this ligand is about two orders of magnitude less than the affinity of 5DII. Thus, if the cellular mechanisms that inactivate 5DI and 5DII are similar, the lesser potency of IOP that inactivates the former enzyme may reflect the fact that higher concentrations are required to interact with the 5DI active site and to initiate the inactivation process.

In addition to this difference in sensitivity, ligand-induced inactivation of 5DI is somewhat slower than that noted for 5DII. In intact GH3 cells, maximal inactivation of 5DII by ligands is noted after a 1–2-h incubation period (23), whereas in the FAO cells used in the present experiments, a 3-h incubation period with rT3 or IOP resulted in only half-maximal inhibitory effects. Of further note is that compared with the 50-min half-life of 5DII activity in GH3 cells and other rat tissues (44), the turnover of 5DI in FAO cells appears to be considerably slower, with only a 5% loss of enzyme activity after a 6-h treatment of cells with cycloheximide.

**Dual mechanism of control of 5DI by iodinated compounds.** The present results demonstrate that iodothyronines and other iodinated compounds have complex regulatory effects on 5DI in the liver and kidney. As demonstrated herein, and previously reported by other investigators (4–6), 5DI is activated by the chronic administration of the metabolically active thyroid hormones T3 or T4. Ligands for the enzyme which have little or no thyromimetic activity (i.e., rT3 and IOP), however, inactivate this enzymatic process and can, when present in high concentrations, counteract the stimulatory effects of T3 on 5DI. Whether this activation and inactivation represent changes in enzyme synthesis and degradation remains uncertain.

Over the last three decades, investigators have observed that the administration of rT3 to patients or laboratory animals, in amounts considerably larger than those used in the present study, counteracts the thyrotoxic state and antagonizes the effects of T4 (45–48). Such treatment has also been demonstrated to decrease hepatic T4 to T3 conversion (49), an effect which has previously been attributed to the competitive effect of rT3 on T3 deiodination that was noted in vitro (10, 50). Recently, Han et al. (51) reported that rT3 also antagonizes the induction of 5DI by insulin, cortisol, and T3 in cultured fetal mouse liver cells. It was uncertain from their study, however, which processes mediated this rT3 effect. The present findings provide a better understanding of the cellular mechanisms that underlie these observations. Our findings do not negate the possibility that rT3, when present in high concentrations, competes in vivo with T4 as a substrate for phenolic ring deiodination. Rather, our results suggest that in so doing, the large ligand load presented by rT3 rapidly inactivates 5DI, and that this is likely to contribute significantly to the impairment observed in T3 production.

**Radiographic contrast agents for the treatment of hyperthyroidism.** Radiographic contrast agents have been used successfully to treat the hyperthyroid state that accompanies Graves’ disease in adults (13, 14, 17). Recent reports have also demonstrated the therapeutic value of administering these agents to patients with neonatal Graves’ disease (52) and thyrotoxicosis factitia (53). IOP and NaI offer several practical advantages when used as treatments for these conditions. They can be administered infrequently (13, 14), are generally well tolerated during chronic therapy (14), and rarely cause hypersensitivity reactions (52). In addition, the rapidity with which serum T3 levels are lowered may have important clinical benefits (17).

The present findings provide new insights into the mechanisms whereby radiographic contrast agents alter thyroid hormone economy in thyrotoxicosis. The inactivating effect of IOP on 5DI in hyperthyroid rats was noted to be rapid in onset and long-lasting in duration. Maximal inactivation was noted within 5 h, and significant effects persisted for 60 h after a single dose despite continued T3 administration. In the clinical setting, the inhibitory effect would be likely to persist even longer, as the rapid lowering of serum and tissue T3 levels would decrease the stimulus for 5DI activation. These results correlate well with clinical observations that serum T3 levels in hyperthyroid patients decrease 60% during the first 24 h of treatment with these agents (15, 16) and that they need be administered only once every 3 d (13).

In a recent study, Wang et al. (54) reported that a fixed dose of IOP (500 mg/d and equivalent to ~1 mg/100 g BW in the patients studied) resulted in the long-term normalization of serum T3 concentrations in only 45% of patients with Graves’ disease. Given the present finding that the inactivating effects of radiographic contrast agents on 5DI are dose-dependent, the therapeutic use of these agents in treating hyperthyroidism may require that the dosage be titrated to achieve optimal effects.

The thyroid gland in patients with Graves’ disease demonstrates increased 5DI activity (36, 37) and enhanced secretion of T3 (55). Indeed, Laurberg (56) has recently demonstrated that both endogenous T4 (that is, T4 derived from thyroglobulin) and circulating T4 are deiodinated to T3 in this organ. The present finding that 5DI in the thyroid gland is also subject to rapid inactivation by IOP provides an explanation for the previous observations that radiographic contrast agents inhibit thyroidal T3 secretion in vivo (57) and 5DI activity in vitro (38, 58).
In conclusion, we have demonstrated that 5'DI is subject to ligand-induced inactivation, an observation which corrects prior misconceptions concerning the mechanism whereby iodothyronines and other iodinated compounds inhibit T4 to T3 conversion in vivo. Although this inactivation process appears to be analogous to that previously delineated for 5'DI, the role of this mechanism in regulating the activity of these two enzymatic pathways appears to differ. Whereas inactivation by substrate is the principal mechanism controlling 5'DI activity (44), it seems unlikely that this process influences 5'DI activity under physiologic or even pathologic conditions; the predominant effect of T4 on 5'DI is one of activation (5, 6, 59) and rT3 concentrations are probably insufficient to exert a significant inactivating effect (49). However, the inactivation of 5'DI is of major pharmacologic importance when the radiographic contrast agents are used to treat hyperthyroid patients. At present, the biochemical processes that mediate the inactivation of the phenolic ring deiodinases remain uncertain. A greater understanding of this unusual mechanism of enzyme regulation is likely to have important theoretical and clinical consequences.

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