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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J Clin Invest. 1988;81(5):1476-1484. https://doi.org/10.1172/JCI113479.

Research Article

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 […]

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 (rT_3) 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 (T_3), 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 rT_3 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 rT_3 and IOP impair thyroxine (T_4) to T_3 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.

Portions of this work appeared in abstract form and were presented at the 69th annual meeting of the Endocrine Society (June 1987, Indianapolis, IN) and the 62nd meeting of the American Thyroid Association (September 1987, Washington, DC).

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Received for publication 8 September 1987 and in revised form 17 November 1987.

J. Clin. Invest.

0021-9738/88/05/1476/09 $2.00
Volume 81, May 1988, 1476–1484

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 3,5,3′-triiodothyronine (T_3)[1] (1). The resultant elevations in the circulating and tissue T_3 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" T_3 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 (T_4) to T_3 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 T_3 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 T_4 to T_3 in the hyperthyroid state.

Radiographic contrast agents such as iopanoic acid (IOP) and sodium ipodate (NaIp) inhibit T_4 to T_3 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 T_3 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 T_3 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 T_3 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

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; rT_3, 3,3′,5′-triiodothyronine; T_3, 3,5,3′-triiodothyronine; T_4, thyroxine; TSH, thyroid-stimulating hormone.

inhibit T_3 neogenesis in vivo. We have recently demonstrated, however, that these agents inhibit T_4 to T_3 conversion in the anterior pituitary gland and cerebral cortex in vivo by noncompetitively 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 T_4 or 3,3',5'-triiodothyronine [reverse T_3, rT_3], 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 sulfhydryl 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'-^{125}I-labeled rT_3 (SA ~ 830 μCi/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, thyroidectomized 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 T_3, rT_3, T_4, 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 $^{125}I^-$ from ^{125}I-rT_3 was measured in the presence of 20 mM dithiothreitol using a modification of the methods of Leonard and Rosenberg (24) and McCann et al. (25). At each concentration of ^{125}I-rT_3, 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 20% TCA solution. Other aliquots were kept at 0–4°C and terminated immediately after adding the ^{125}I-rT_3 to determine basal $^{125}I^-$ content. The $^{125}I^-$ 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 $^{125}I^-$ 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'-diiodothyronine and I^- were produced from ^{125}I-rT_3 in the homogenates of kidney, liver, and thyroid tissue. No $^{125}I^-$ was released in tissue-free incubations performed at 37°C. 5'DI activity was expressed as picomoles of $^{125}I^-$ released per minute per milligram protein after multiplying by a factor of two to correct for the random labeling of ^{125}I-rT_3 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 K_i was calculated by replotting the slope of the double reciprocal plot vs. the concentration of competitive inhibitor. On such a plot, the K_i 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-cm^2 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 transferred and maintained for 24–48 h in serum-free RPMI 1640 medium before experimentation. 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 (Artek 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'-T_2 and I^- were produced from ^{125}I-rT_3 in the FAO cell sonicates.

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

Ascending 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 (3:20:40 vol/vol). R_f values for the iodinated compounds of interest were: 0.31 for rT_3, 0.42 for 3,3'-T_2, and 0.67 for I^-.

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 intersected at the y-axis on a double reciprocal plot (Fig. 1). Slope-replots of such data (Fig. 1, inset) from two experiments yielded K_i 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 revealed a decrease in V_{max} with no change in the value of the K_m, 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_m values were unchanged 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 inhibition of ^{125}I-rT_3 deiodination.

The effects of the chronic administration of T_3, rT_3, and IOP on the regulation of 5'DI in the kidney and liver were examined in a second group of thyroidectomized rats (Fig. 3). Twice daily, injections of rT_3 (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 T_3 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 rT_3 and IOP to antagonize the stimulating effects of T_3 were also examined (Fig. 3). 5'DI activity in hyperthyroid animals was unchanged after a single injection of rT_3 (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 resulted in levels of 5'DI activity that were less than or similar to

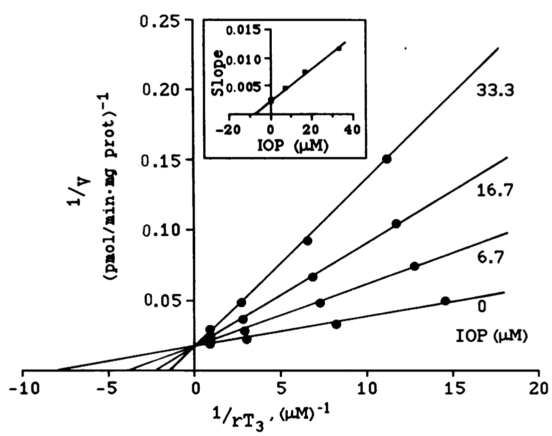

Figure 1. Effects of IOP on 5'DI activity in a broken kidney cell preparation. A kidney homogenate was prepared from a euthyroid animal and the diluted supernatant fraction was used to determine 5'DI activity. IOP, in the final concentrations indicated, was added to aliquots of the assay mixture before the quantitation of enzyme activity. A double reciprocal plot of the kinetic data demonstrates a competitive pattern of inhibition. A replot of the slope of the double reciprocal plots vs. the IOP concentration (*inset*) demonstrated a K_i value of 8 μM. Similar results were obtained in a second experiment.

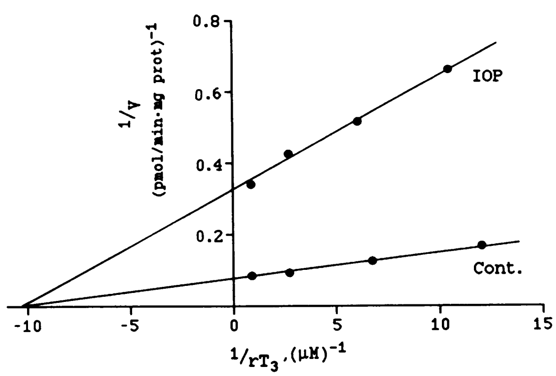

Figure 2. Effect of the in vivo administration of IOP on kidney 5'DI activity. Thyroidectomized rats were given an intraperitoneal injection of IOP (4 mg/100 g BW) or vehicle (control) and killed 3 h later. Kidney homogenates from individual animals were then prepared and 5'DI activity was determined in the diluted supernatant fractions. A double reciprocal plot of kinetic data from two representative animals demonstrates a noncompetitive pattern of inhibition.

those noted in euthyroid control rats. Again, K_m values in all groups were unchanged (Fig. 3), which suggests that the observed 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_m value is equal to the negative value of the slope (26).

The time course of the in vivo inhibitory effects of IOP on 5'DI in hyperthyroid animals was investigated in another experiment in which rats were treated chronically with T_3 (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, T_3 administration was continued until 12 h before killing. Hyperthyroid animals again demonstrated significantly higher 5'DI activity in the liver and kidney when compared 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

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

Treatment	n*	V_{max}	K_m
		pmol/min·mg protein	*μM*
Control	6	13.3±0.7	0.10±0.01
IOP (0.04)‡	5	6.4±0.6§	0.10±0.01
IOP (4)	5	4.5±0.5§	0.11±0.03

* n, number of rats.
‡ IOP, in a single dose of 0.04 or 4 mg/100 g BW, i.p., was administered 3 h before killing to rats that had been thyroidectomized 8 wk earlier. Data represent the mean±SE.
§ $P < 0.001$ vs. control (Dunnett's t test).

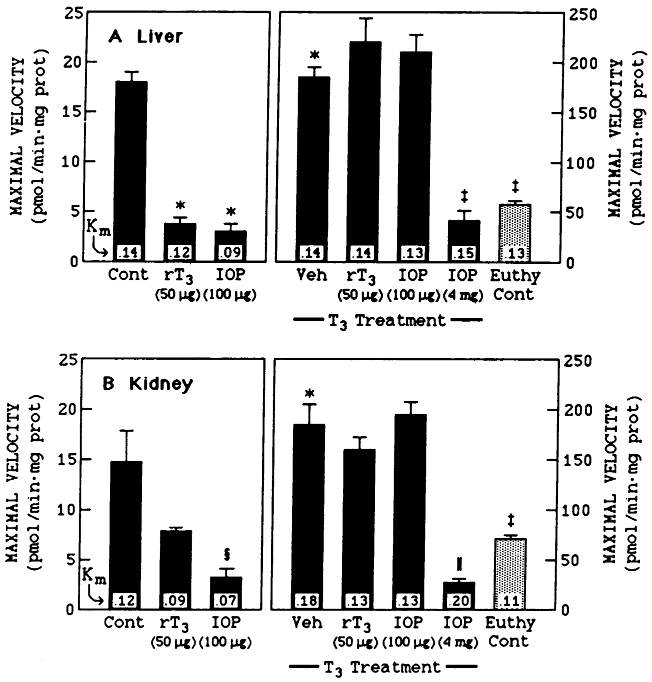

Figure 3. Effects of the chronic and acute in vivo administration of rT_3, IOP, and T_3 on 5'DI activity in the (*A*) liver and (*B*) kidney. Thyroidectomized rats received twice daily subcutaneous injections for 4 d according to the following protocol: (*a*) control, vehicle solution; (*b*) rT_3, 50 µg/100 g BW; (*c*) IOP, 100 µg/100 g BW; (*d*) T_3 treatment, 0.6 µg/100 g BW. Animals were killed 12 h after the last injection and 5'DI activity was quantified in diluted supernatant fractions of liver and kidney homogenates. 6 h before killing, T_3-treated animals received a single intravenous injection of vehicle (veh), rT_3 (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 K_m value of each group expressed in µmol/liter. *$P < 0.01$ vs. thyroidectomized control group; ‡$P < 0.01$ vs. T_3 vehicle treatment group; §$P < 0.05$ vs. thyroidectomized control; ||$P < 0.01$ vs. T_3 vehicle treatment group and vs. euthyroid control group.

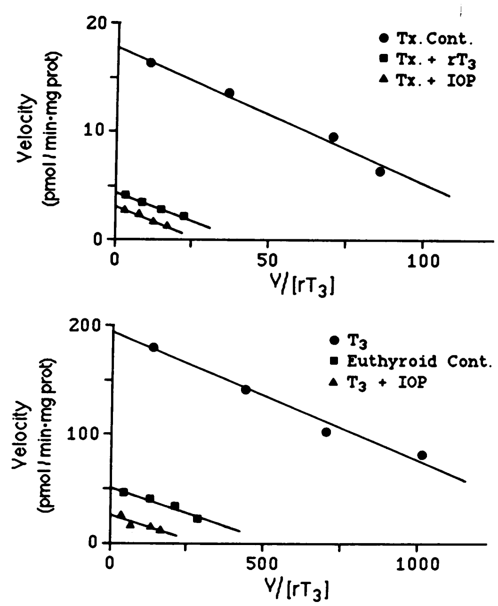

Figure 4. Hepatic 5'DI activity, as analyzed by Eadie-Hofstee plots, of representative, individual animals from the experiment depicted in Fig. 3. On these plots, the *y*-intercept represents the V_{max} value, and the negative value of the slope indicates the K_m. A noncompetitive pattern of inhibition is demonstrated. Concentrations of rT_3 on the abscissa are expressed in µmol/liter. Tx, thyroidectomized.

the IOP injection. Although partial recovery of 5'DI activity was noted at the 60-h time point, V_{max} 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'DI activity in the thyroid gland. 5'DI 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'DI V_{max} 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'DI 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 V_{max} levels at both time points. At 5 h after the IOP injection, 5'DI 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 K_m values in either treatment group. Thus, as is the case in the liver and the kidney, radiographic contrast agents inhibit 5'DI in the thyroid gland in vivo by a noncompetitive mechanism.

Regulation of 5'DI 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'DI activity was investigated. Phenolic ring deiodinase activity was easily demonstrated in FAO cell sonicates using ^{125}I-rT_3 as substrate. Characterization of this deiodinating process revealed the following: (*a*) $^{125}I^-$ release from ^{125}I-rT_3 was thiol-dependent with maximal product formation noted at DTT concentrations of 5–20 mM; (*b*) no $^{125}I^-$ production was noted in cell sonicates incubated at 0–4°C or in sonicates previously heated to 80°C for 30 min; (*c*) $^{125}I^-$ 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 ^{125}I-rT_3; (*e*) in the presence of 20 mM dithiothreitol, PTU at a concentration of 1 mM inhibited $^{125}I^-$ production by > 99%; (*f*) using 1 nM ^{125}I-T_3 as a substrate, no phenolic or tyrosyl ring deiodinating activity could be demonstrated in cell sonicates under routine assay conditions; (*g*) $^{125}I^-$ 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 ^{125}I-rT_3 as substrate in sonicates prepared from FAO cells that were maintained in serum-free medium, saturable reaction kinetics were demonstrated for phenolic ring

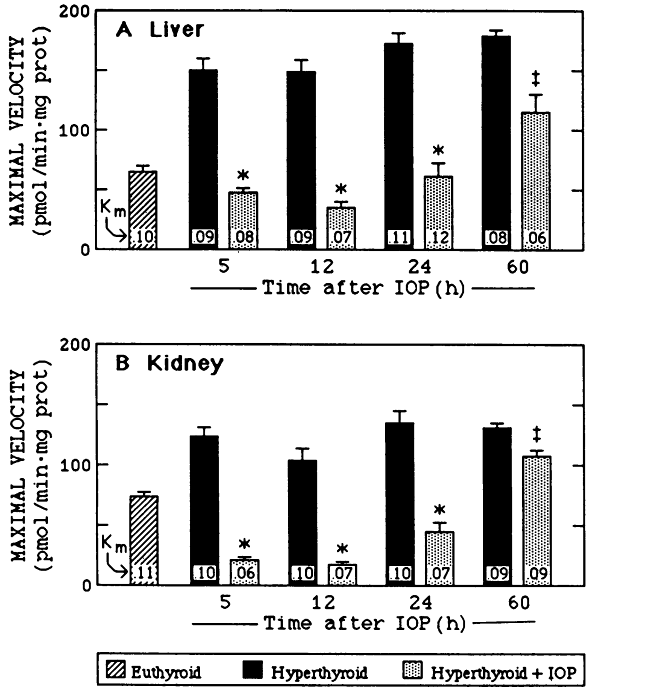

Figure 5. Time course of the inhibitory effect of IOP on 5'DI activity in the (*A*) liver and (*B*) kidney of hyperthyroid rats. Euthyroid rats were rendered hyperthyroid by the subcutaneous injection of T_3 (1 μg/100 g BW twice daily) for 4 d. The hyperthyroid rats were then administered a single, intravenous dose of vehicle solution or IOP (4 mg/100 g BW) and killed 5, 12, 24, or 60 h later. T_3 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.

Table II. Effects of the In Vivo Administration of IOP on 5'DI Activity in the Thyroid Gland of MMI-Treated Rats

Treatment	n*	V_{max}	K_m
		pmol/min · mg protein	μM
Euthyroid	7	163±13	0.09±0.01
MMI control‡	6	137±10	0.12±0.01
MMI + IOP (5 h)	5	97±12§	0.11±0.01
MMI + IOP (12 h)	5	66±7‖	0.12±0.02

* *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 Dunnett's *t* test.

deiodinase activity. In seven experiments, mean K_m and V_{max} values were 0.22±0.02 μM and 5.3±1.1 pmol $^{125}I^-$ 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 rT_3 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_{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_{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 rT_3 were approximately equipotent to those of IOP. V_{max} values were decreased by 36±6, 46±8, and 90±1% in three experiments in which cells were incubated with rT_3 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 rT_3; 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 rT_3 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 [^{3}H]leucine incorporation into TCA-precipitated 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 rT_3 and IOP on 5'DI activity. The addition of rT_3 (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 GH_3 pituitary tumor cells is that exposure of intact

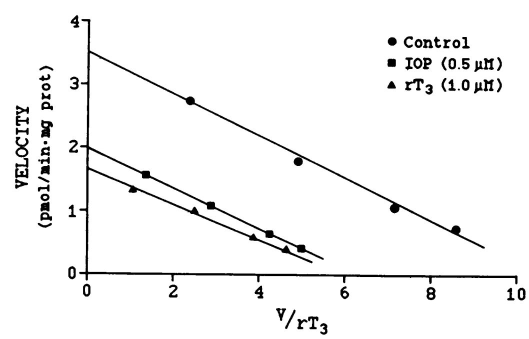

Figure 6. Effects of IOP and rT_3 on 5'DI activity in intact, cultured Reuber FAO hepatoma cells. FAO cells were grown to confluence in serum-containing medium, transferred, and maintained for 24 h in serum-free RPMI 1640 medium. IOP or rT_3, in the final concentrations shown, were then added to the 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 rT_3 on the abscissa are expressed in μmol/liter.

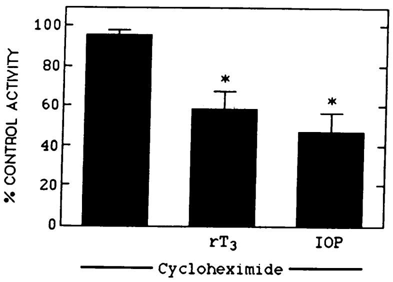

Figure 7. The effects of rT_3 and IOP on 5'DI activity in intact, cultured FAO cells treated with cycloheximide. FAO cells were grown to confluence in serum-containing medium and then transferred and maintained for 24 h in serum-free RPMI 1640 medium. Cycloheximide (50 μg/ml), with or without rT_3 (1 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 sulfhydryl oxidizing agent diamide results in a rapid inactivation of 5'DII activity which mimicks the effects of rT_3, 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 V_{max} levels decreased by 75±4% ($P < 0.025$), whereas K_m 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

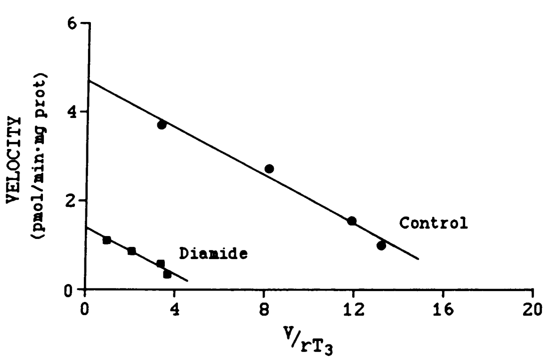

Figure 8. Effect of diamide on 5'DI activity in intact, cultured FAO hepatoma cells. FAO cells were grown to confluence in serum-containing medium and then transferred and maintained for 24 h in serum-free RPMI 1640 medium. The medium was then changed to serum-free and glucose-free RPMI 1640, with or without diamide (0.4 mM), and the cells incubated for an additional 3.5 h. Cells were then washed and harvested with buffers containing 5 mM DTT and 5'DI activity was determined in the supernatant fractions of the cell sonicates. The data depicted are Eadie-Hofstee plots from a single representative experiment. A noncompetitive pattern of inhibition is demonstrated. Concentrations of rT_3 on the abscissa are expressed in μmol/liter.

(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 T_4 to T_3 (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'DI activity (5, 6), the activity of 5'DII 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., rT_3 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 V_{max} with no change in K_m. 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'DI present in FAO cells, rT_3 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 T_4 to T_3 by intact, cultured rat hepatocytes. Their studies, however, did not determine whether the decrease in T_3 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 GH_3 cells to sulfhydryl oxidizing agents rapidly inactivates the enzyme, whereas exposure to sulfhydryl 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'DI process appears to be considerably less sensitive than the 5'DII 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 kidney (Fig. 3). Indeed, a 70–80% inhibition of 5'DI activity is only achieved when hypothyroid or hyperthyroid rats are administered a 100-fold larger dose of IOP (Table I and Fig. 3). Similarly, a single dose of rT_3 of 50 μg/100 g BW, which has been demonstrated by other investigators to inhibit 5'DII activity in the pituitary gland and cerebral cortex by > 90% (43), had no effect on 5'DI activity when administered to hyperthyroid animals in the present studies. Results from our cell culture studies provide additional evidence that 5'DI is less sensitive to ligand-induced inactivation. We have previously demonstrated that the concentration of IOP required to inhibit 5'DII activity by 50% in intact GH_3 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 5'DI 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 5'DI as compared with 5'DII. 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 5'DII in intact GH_3 cells is closely correlated with the ligand's K_i value as determined in broken cell preparations and indicative of the affinity of the ligand for the enzyme's active site (23). The K_i values of IOP for 5'DI and 5'DII are 8 μM (Fig. 1) and 0.05 μM (23), respectively, and therefore the affinity of 5'DI for this ligand is about two orders of magnitude less than the affinity of 5'DII. Thus, if the cellular mechanisms that inactivate 5'DI and 5'DII 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 5'DI active site and to initiate the inactivation process.

In addition to this difference in sensitivity, ligand-induced inactivation of 5'DI is somewhat slower than that noted for 5'DII. In intact GH_3 cells, maximal inactivation of 5'DII 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 rT_3 or IOP resulted in only half-maximal inhibitory effects. Of further note is that compared with the 50-min half-life of 5'DII activity in GH_3 cells and other rat tissues (44), the turnover of 5'DI 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 5'DI by iodinated compounds. The present results demonstrate that iodothyronines and other iodinated compounds have complex regulatory effects on 5'DI in the liver and kidney. As demonstrated herein, and previously reported by other investigators (4–6), 5'DI is activated by the chronic administration of the metabolically active thyroid hormones T_3 or T_4. Ligands for the enzyme which have little or no thyromimetic activity (i.e., rT_3 and IOP), however, inactivate this enzymatic process and can, when present in high concentrations, counteract the stimulatory effects of T_3 on 5'DI. 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 rT_3 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 T_4 (45–48). Such treatment has also been demonstrated to decrease hepatic T_4 to T_3 conversion (49), an effect which has previously been attributed to the competitive effect of rT_3 on T_4 deiodination that was noted in vitro (10, 50). Recently, Han et al. (51) reported that rT_3 also antagonizes the induction of 5'DI by insulin, cortisol, and T_3 in cultured fetal mouse liver cells. It was uncertain from their study, however, which processes mediated this rT_3 effect. The present findings provide a better understanding of the cellular mechanisms that underlie these observations. Our findings do not negate the possibility that rT_3, when present in high concentrations, competes in vivo with T_4 as a substrate for phenolic ring deiodination. Rather, our results suggest that in so doing, the large ligand load presented by rT_3 rapidly inactivates 5'DI, and that this is likely to contribute significantly to the impairment observed in T_3 production.

Radiographic contrast agents for the treatment for 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 NaIp 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 T_3 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 5'DI 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 T_3 administration. In the clinical setting, the inhibitory effect would be likely to persist even longer, as the rapid lowering of serum and tissue T_3 levels would decrease the stimulus for 5'DI activation. These results correlate well with clinical observations that serum T_3 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 T_3 concentrations in only 45% of patients with Graves' disease. Given the present finding that the inactivating effects of radiographic contrast agents on 5'DI 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 5'DI activity (36, 37) and enhanced secretion of T_3 (55). Indeed, Laurberg (56) has recently demonstrated that both endogenous T_4 (that is, T_4 derived from thyroglobulin) and circulating T_4 are deiodinated to T_3 in this organ. The present finding that 5'DI 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 T_3 secretion in vivo (57) and 5'DI 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 T_4 to T_3 conversion in vivo. Although this inactivation process appears to be analogous to that previously delineated for 5'DII, 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'DII activity (44), it seems unlikely that this process influences 5'DI activity under physiologic or even pathologic conditions; the predominant effect of T_4 on 5'DI is one of activation (5, 6, 59) and rT_3 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.

Acknowledgments

The author thanks Ms. Janet Fatherly for her secretarial assistance, Dr. Lee Witters for his advice and support, and Dr. Valerie Anne Galton for her critical review of the manuscript.

This investigation was supported by a grant from the Hitchcock Foundation (Hanover, NH). The author is the recipient of a Clinical Investigator Award (DK-01389) from the National Institute of Diabetes and Digestive and Kidney Diseases.

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