Cerebral Cortex Responds Rapidly to Thyroid Hormones

Abstract. *In rats subjected to thyroidectomy there was a two- to fourfold increase in cerebral cortex iodothyronine 5′-deiodinase activity within 24 hours. This increase was prevented by thyroxine replacement. The increased cortical 5′-deiodinase in chronically hypothyroid rats was normalized within 4 hours by a single intravenous injection of triiodothyronine. These results indicate that the adult central nervous system can give a very rapid biochemical response to thyroid hormone.*

Thyroid hormones are essential for normal development of the mammalian central nervous system. Neonatal hypothyroidism results in multiple morphological and functional abnormalities in man and in experimental animals (*1*). Adults with severe thyroid hormone excess are characteristically tremulous and show emotional lability, whereas patients with chronic thyroid hormone deficiency sometimes develop memory impairment, deafness, cerebellar ataxia, or even coma. Despite these obvious functional abnormalities, it has been difficult to demonstrate that specific metabolic processes in the brain, including the rate of oxygen consumption, are thyroid hormone dependent (*2, 3*). Nonetheless, like other thyroid hormone responsive tissues, the adult rat brain contains significant amounts of intracellular thyroid hormone (*4*) and specific thyroid hormone nuclear receptors (*5*).

Rat cerebral cortex tissue deiodinates thyroxine (T_4) to form the more active thyroid hormone 3,5,3′-triiodothyronine (T_3) in vivo (*6*) and in vitro (*7*), a process termed 5′-deiodination. This tissue also inactivates thyroid hormone by removal of a tyrosyl ring iodine (*7, 8*), a process termed 5-deiodination. Crantz and Larsen (*9*) estimate that more than 70 percent of the intracellular T_3 found in cerebral cortex is derived from the intracellular 5′-deiodination of T_4. Kaplan and Yaskoski (*7*) demonstrated increased 5′-deiodinase and decreased 5-deiodinase in homogenates of the cerebral cortex of chronically hypothyroid rats indicating that this tissue does respond, directly or indirectly, to changes in thyroid status (*7, 8*). The present studies were performed to discover the time course of these changes in iodothyronine metabolism in response to alterations in plasma thyroid hormones. The results indicate that within 24 hours of thyroidectomy there is a two- to fourfold increase in iodothyronine 5′-deiodinase in the rat cerebral cortex. This change is followed within 4 days by a decrease in iodothyronine 5-deiodinase activity. The elevated cerebral cortex 5′-deiodinase activity in chronically hypothyroid rats is reduced to normal within 4 hours by T_3. To our knowledge, this is the first demonstration of a very rapid biochemical response of the adult central nervous system to thyroid hormone.

Male Sprague-Dawley rats weighing 175 to 200 g were surgically thyroidectomized under ether anesthesia or were subjected to sham operations. Thyroidectomized rats with parathyroid implants were also obtained from Zivic-Miller and kept for 1 to 3 months before use. At indicated times after thyroidectomy, groups of four to eight animals were lightly anesthetized with ether and exsanguinated via the abdominal aorta. The cerebral cortex from individual rats was homogenized in ten volumes (weight to volume) of 0.32*M* sucrose containing 10 m*M* Hepes buffer (*p*H 7.0) and 10 m*M* dithiothreitol (DTT) at 4°C. Iodothyronine 5′-deiodinase activity was determined in cerebral cortex homogenates by measuring the radioactive iodide liberated from reverse T_3 (rT_3; that is, 3,3′,5′-triiodothyronine) singly labeled in the distal ring (*10*), at tissue dilutions showing a linear relation between protein concentration and product formation. Deiodination products in acid extracts of the incubation mixtures were separated by ion-exchange chromatography on Dowex 50W-X8 (*10, 11*). The conversion of T_4 to T_3 in cerebral cortex homogenates was also determined (*7, 8*). Iodothyronine 5-deiodinase activity was determined by measuring the formation of ^{125}I-labeled 3,3′-diiodothyronine (3,3′-

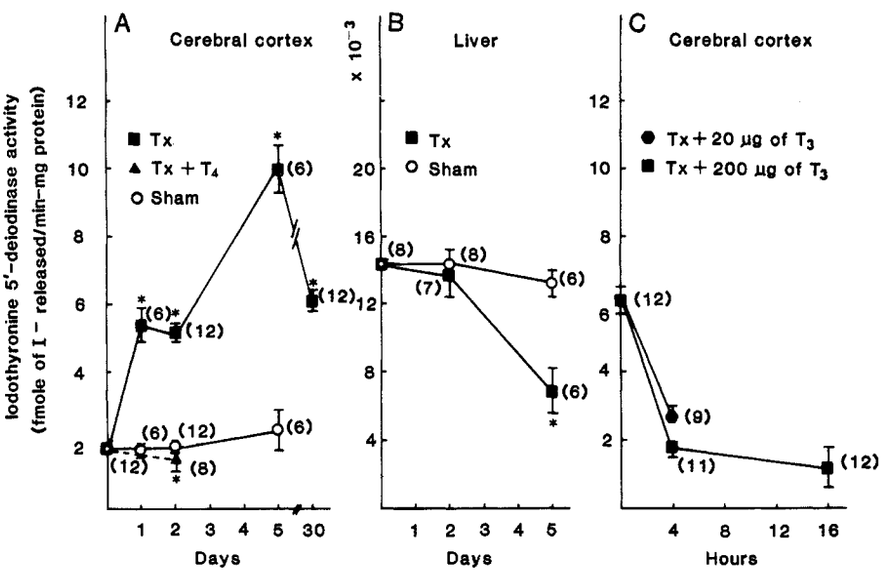

Fig. 1. Effects of thyroidectomy and thyroid hormone treatment on cerebral cortex and hepatic iodothyronine 5′-deiodination. (A) Time course of changes in cerebral cortex 5′-deiodination after thyroidectomy. Cerebral cortex homogenates from individual rats were diluted fourfold with 320 m*M* sucrose, 10 m*M* Hepes buffer (*p*H 7.0), 10 m*M* DTT (dilution factor, 1:40, wet weight of tissue to volume), and 50 μl of this tissue suspension was added to 50 μl of substrate mixture containing 10 μmole of potassium phosphate buffer (*p*H 7.0), 0.1 μmole of EDTA, 1.5 μmole of DTT, and 1 pmole of ^{125}I-labeled rT_3 (100 counts per minute per femtomole). Incubations were performed in triplicate, at 37°C for 45 minutes under N_2. Reactions were terminated by adding 50 μl of serum containing 10 m*M* 6-*n*-propylthiouracil and then by 350 μl of 10 percent trichloroacetic acid (TCA). After centrifugation, 400-μl portions of the TCA soluble material were applied to 1-ml columns of Dowex 50W-X8 equilibrated in 10 percent acetic acid. Iodide was eluted with two 1-ml portions of 10 percent acetic acid and the radioactivity in the combined eluates was determined. For the protein determinations we used human immunoglobulin G as the standard (*17*). (B) Time course of changes in hepatic 5′-deiodination after thyroidectomy. Livers from individual rats were homogenized separately in ten volumes (wet weight to volume) of 320 m*M* sucrose, 10 m*M* Hepes buffer (*p*H 7.0), and 1 m*M* DTT and were diluted an additional ten times before being assayed for enzyme activity. Reaction mixtures contained, in 100 μl, 10 μmole of potassium phosphate buffer, 0.1 μmole of EDTA, 0.1 μmole of DTT, 100 pmole of rT_3 (500 counts per minute per picomole), and tissue. Reactions were started by the addition of 25-μl portions of the diluted hepatic homogenates and allowed to proceed at 37°C for 10 minutes. Reaction tubes were then treated as before. Note that reaction rates in the liver are $\approx 10^3$ times those in the cerebral cortex. (C) Effects of T_3 administration on cerebral cortex 5′-deiodination in chronically hypothyroid rats. Tissue preparations and reaction conditions were as described for (A). At each data point the mean ± standard error is shown; the number of animals per group is indicated in parentheses. Asterisk indicates $P < .001$; *Tx*, thyroidectomized rats.

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T_2) from ^{125}I-labeled T_3 (*7, 8*). Serum T_4, T_3, and thyroid stimulating hormone (TSH) were measured by specific radioimmunoassay according to established methods (*12*).

The effects of thyroid hormone deprivation on cerebral cortex 5′-deiodinase activity are depicted in Fig. 1A. Enzyme actvity in cerebral cortex increased within 1 day of thyroidectomy to 2.9 times that in controls, and was 4.3 times that of the animals with sham operations at 5 days. The activity of 5′-deiodinase, as judged by T_4 to T_3 conversion in cortex homogenates, was also increased five, four, and seven times at 1, 2, and 5 days after thyroidectomy, respectively. The activity 5 days after thyroidectomy appeared to be higher than the activity in chronically hypothyroid rats (Fig. 1A). At 1 day after thyroidectomy the serum T_4 and T_3 concentrations were significantly decreased: 1.0 ± 0.2 (standard error of the mean) compared to 3.9 ± 0.4 μg of T_4 per deciliter and 28 ± 2 compared to 75 ± 3 ng of T_3 per deciliter; and serum TSH was increased (780 ± 119 compared to 242 ± 42 μU of TSH per milliliter). Serum hormone concentrations continued to decrease in the thyroidectomized rats so that 5 days after surgery the concentration of serum T_4 was < 0.3 μg/dl and of serum T_3 was 18 ± 3 ng/dl.

If one assumes a cerebral cortical T_4 content of about 2.5 ng per gram of wet weight in euthyroid rats (*4*), the concentration of endogenous T_4 contained in the 1:80 dilution of the homogenate from euthyroid rats was $4 \times 10^{-11} M$. Addition of this amount of T_4 to the cortical homogenates of thyroidectomized rats had no effect on the rate of 5′-deiodination, indicating the increased enzyme activity was not a simple consequence of the decrease in the homogenate T_4 concentrations.

The activity of 5-deiodinase in cerebral cortex was unchanged 1 and 2 days after thyroidectomy but showed a decrease of 54 ± 16 percent (mean ± standard error) after 5 days. Similarly, hepatic 5′-deiodinase in the same animals was unchanged in the thyroidectomized group after 2 days but was decreased to 48 percent of control after 5 days (Fig. 1B).

The increase in cerebral cortex 5′-deiodinase activity that was found consistently 48 hours after thyroidectomy was prevented by subcutaneous injection of physiological replacement quantities of T_4 (800 ng per 100 g of body weight) 8 and 32 hours after surgery (Fig. 1A). Animals treated with these quantities of T_4 had both normal serum TSH and T_4 concentrations (T_4 = 4.1 ± 0.2; TSH = 285 ± 53) 48 hours after thyroidectomy, as was observed previously (*13*). In control rats that received sham operations this quantity of T_4 had no effect on cerebral cortex deiodinating enzymes. Since changes in cerebral cortex deiodination could be a direct effect of T_4, as opposed to an effect of the T_3 derived from it, chronically hypothyroid rats were given several T_3 treatment regimens. A single intravenous injection of 200 μg of T_3 per 100 g 16 hours before the rats were killed (Fig. 1C), which would saturate all nuclear T_3 receptors in the cerebral cortex for this period (*14*),

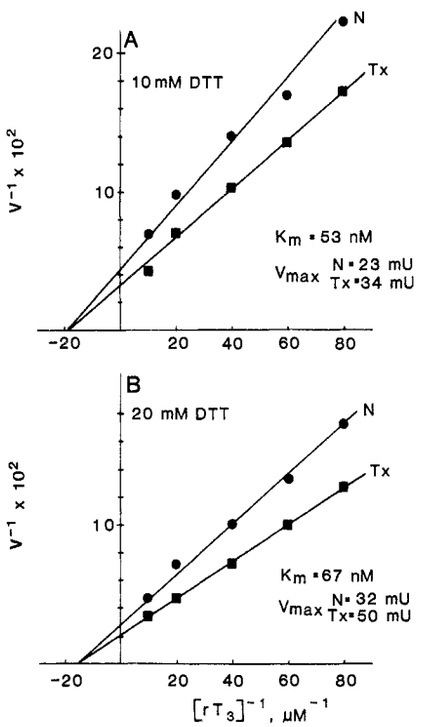

Fig. 2. Double reciprocal plots of reaction velocity versus rT_3 concentrations for iodothyronine 5′-deiodinase activity in cerebral cortex microsomal preparations from control (*N*) and thyroidectomized (*Tx*) rats. Cerebral cortex from rats subjected to sham operations or thyroidectomy 2 days previously served as sources of enzyme activity. Ten percent homogenates (see Fig. 1A) were centrifuged at $20{,}000 g_{max}$ for 20 minutes and the supernatant was further centrifuged at $100{,}000 g_{max}$ for 60 minutes. The resultant pellets were suspended in homogenizing buffer (2 mg of protein per milliliter). Portions (50 μl) of the microsomal suspensions were then incubated with increasing concentrations of rT_3 (10 to 100 n*M*) in 100 m*M* potassium phosphate buffer (*p*H 7.0) at 10 and 20 m*M* DTT for 45 minutes at 37°C under N_2. Reaction tubes were processed as before (see Fig. 1). Each data point represents the mean of closely (± 10 percent) agreeing triplicate determinations. 1U = 1 picomole of I^- per minute per milligram of protein.

decreased cerebral cortex 5′-deiodinase activity to euthyroid levels. A decrease in the duration of exposure to T_3 as well as a tenfold reduction in the amount of T_3 injected did not significantly alter the results: both 20 and 200 μg of T_3 given 4 hours before the rats were killed significantly ($P < .001$) decreased cerebral cortex 5′-deiodinase in chronically hypothyroid animals. In all of these experiments, significant decreases in T_4 to T_3 conversion were also observed, whereas little or no change in 5-deiodinase was demonstrated. These data indicate that both T_4 and T_3 can modulate iodothyronine 5′-deiodinase in cerebral cortex.

Reaction kinetics were determined in crude membrane preparations of cerebral cortex from rats that had been thyroidectomized or given sham operations 2 days previously. Microsomal membranes prepared by differential centrifugation were incubated with increasing concentrations of rT_3 at 10 m*M* and 20 m*M* DTT. As judged from double reciprocal plots of the data (Fig. 2), thyroid hormone deficiency increased the maximum velocity of the deiodination reaction without altering the apparent Michaelis constants. Increases in DTT in the reaction mixture caused a parallel downward displacement of the double reciprocal plots (compare the slopes of the lines for the 5′-deiodinase activity at 10 and 20 m*M* DTT). These results suggest that thyroid hormone deficiency results in an increase in the number of enzyme units in the cerebral cortex and that the initial velocity reaction kinetics of the 5′-deiodination of rT_3 in this tissue are of "Ping-Pong" type, as has been reported for this reaction in liver and kidney (*10, 15*). Whether the increase in 5′-deiodinase is due to the activation, recruitment, or alteration in the synthesis or degradation of the enzyme (or enzymes) remains to be established.

The rapid changes in the activity of cerebral cortex iodothyronine 5′-deiodinase in response to alterations in the concentrations of circulating thyroid hormone are surprising. The capacity for such an immediate biochemical response to thyroidectomy was previously thought to be unique to the anterior pituitary gland, as manifested by a rapid increase in thyrotropin secretion and in the conversion of T_4 to T_3 (*16*). The reciprocal relation between serum T_4 concentrations and cerebral cortex enzyme activity illustrate the potential role for compensatory changes in intracellular iodothyronine metabolism (*16*). These changes could serve to defend the intracellular T_3 of the cerebral cortex

against reductions in the plasma T_4, thereby blunting effects of hypothyroidism in this tissue.

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Persistent Behavior at High Rates Maintained by Intravenous Self-Administration of Nicotine

Abstract. *Squirrel monkeys pressed a lever at high rates under a second-order schedule of reinforcement in which lever pressing produced a brief visual stimulus that was occasionally contiguous with an intravenous injection of nicotine. The rate of lever pressing could be markedly reduced either by substituting saline for nicotine injections or by blocking the effects of nicotine with mecamylamine. The rate of lever pressing could also be reduced by eliminating the brief visual stimulus. These results show that nicotine can function as an effective reinforcer under a second-order schedule of drug self-administration and that an environmental stimulus associated with nicotine intake can contribute to the maintenance of persistent drug-seeking behavior.*

The role of nicotine in the maintenance of tobacco smoking has been questioned because of difficulties in demonstrating consistent reinforcing effects of the drug under controlled laboratory situations. Variations in the nicotine content of cigarettes or treatment with agents that block nicotine's actions have occasionally been found to alter human smoking behavior, but the changes have been small, not always reproducible, and open to disparate interpretations (*1*). Moreover, reliable evidence that nicotine can function as a reinforcer of animal drug self-administration is limited. Some investigators, including ourselves, have reported that intravenous injections of nicotine can maintain self-administration behavior of rats or monkeys, but the levels of responding usually have been low (*2*, *3*). Other investigators have found nicotine to be ineffective in maintaining self-administration behavior (*4*).

If nicotine functions as a reinforcer to maintain tobacco smoking, it is likely that its reinforcing effects are magnified by interactions with interoceptive and exteroceptive stimuli associated with smoking, such as taste, tactile sensation, and social setting (*1*). The temporal contiguity of these stimuli with the reinforcing effects of nicotine may result in the stimuli's acquiring conditioned reinforcing properties, which further strengthen smoking behavior. Consequently, nicotine might function more effectively to maintain self-administration behavior by laboratory animals if it were studied under conditions in which responding resulted not only in nicotine injections but also in presentations of environmental stimuli associated with injections.

Previous studies have shown that long and orderly sequences of responding can be maintained by scheduled presentations of environmental stimuli that have been associated with intravenous injections of drugs such as morphine and cocaine (*5*, *6*). The schedules of reinforcement relating responding to consequent presentations of the stimuli and injections of drugs in these studies have been termed second-order schedules (*5*, *6*). We now report that intravenous injections of nicotine can maintain very high rates of lever-press responding by squirrel monkeys under a second-order schedule in which responding results in presentations of a visual stimulus that is intermittently associated with nicotine injection.

Four mature male squirrel monkeys (*Saimiri sciureus*) had venous catheters permanently implanted (*7*) and had unrestricted access to food and water in their individual living cages. During experimental sessions, the monkeys sat in a chair equipped with a response lever and green and amber stimulus lights (*8*); the chair was enclosed in a sound-attenuating chamber. Injections were delivered through the catheters from an infusion pump located outside the chamber (*9*). Before the experiment began, three monkeys (S-151, S-156, and S-200) had been trained to press a lever under a second-order schedule of intravenous cocaine injection (*10*); responding was subsequently extinguished by substituting saline for drug injections. The fourth monkey (S-464) was experimentally naïve at the beginning of the study.

In the cocaine-trained monkeys, responding was established under a second-order schedule of intravenous nicotine injection without preliminary training. In the untrained monkey (S-464), responding was first established under a fixed-interval (FI) schedule of intravenous nicotine injection (*11*), and the schedule then was changed to a second-order schedule. Under the second-order schedule, the green stimulus light was

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