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THE EFFECTS OF THYROID STATE ON BETA-ADRENERGIC AND SEROTONERGIC RECEPTORS IN RAT BRAIN

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SUMMARY

The effects of hyperthyroidism or hypothyroidism, alone or in combination with the tricyclic antidepressant desmethylimipramine (DMI), on brain beta-adrenergic and serotonin (5HT₂) receptors were studied in adult male Sprague–Dawley rats. Intraperitoneal administration of T₃ or T₄ for 7 days increased the number of cortical beta-adrenergic and 5HT₂ receptors. These increases were significant at levels of 250 µg/kg or above for both hormones. Neither thyroidectomy nor “reverse” T₃ (rT₃) (500 µg/kg) produced an effect on either receptor type. The down-regulation of beta-adrenergic receptors produced by daily subcutaneous injections of 20 mg/kg of DMI for 7 days was partially offset by concurrent administration of T₄, whereas the down-regulation of 5HT₂ receptors produced by the drug was not affected by concurrent administration of either T₃ or T₄. Hypothyroidism (thyroidectomy) did not significantly affect the adaptation of these receptor populations to DMI.

As regards brain regions other than cortex, T₄ (250 µg/kg) produced the same changes in hippocampus as in cortex, while thyroidectomy decreased beta-adrenergic receptors only in the cerebellum. Thyroxine also elevated 5HT₂ receptors in the hippocampus; thyroidectomy caused a significant decrease in 5HT₂ receptors in the striatum.

INTRODUCTION

THE INFLUENCE of endocrine status on behaviors and affect is exemplified by the high incidence of lethargy, confusion and depressive symptoms in clinical hypothyroidism. Clinical depression occurs in about 40% of hypothyroid patients, and in most of these patients replacement therapy produces prompt remission of depressive symptoms (Whybrow *et al.*, 1969, 1972; Sachar, 1975; Whybrow and Prange, 1981). In depressed patients activation of the thyroid axis, as indicated by increased secretion of thyroid hormones, is predictive of a rapid response to therapy with a tricyclic antidepressant (TCA) (Whybrow and Prange, 1981). Furthermore, concurrent administration of L-triiodothyronine (T₃) with a TCA has been found to accelerate the antidepressant effect in women (Prange *et al.*, 1969; Wilson *et al.*, 1970; Wheatly, 1972; Coppen *et al.*, 1972) and to produce a therapeutic response in patients of both sexes who previously did not respond to TCA treatment alone (Earle, 1970; Hatotani *et al.*, 1974; Ogura *et al.*, 1974; Goodwin *et al.*, 1982). The notion that thyroid hormones affect brain function is strengthened by evidence that in rats thyroid hormones are taken up into the synaptosomal fraction of brain neurons, and that L-thyroxine (T₄) is converted there to its more potent metabolite T₃ (Dratman and Crutchfield, 1978). Furthermore, it has been demonstrated that the control and kinetics of

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the 5'-monodeiodinase which converts T_4 to T_3 , found in almost every peripheral organ (type I), is different from the enzyme (type II) present in brain, brown fat and pituitary (Visser *et al.*, 1982). These enzymatic differences may be responsible for the marked differences in changes which occur in central and peripheral levels of T_3 during starvation, for example, in which peripheral T_3 is reduced and brain T_3 relatively unchanged (Larsen *et al.*, 1981).

Beginning with what is now called the catecholamine hypothesis (Prange, 1964; Schildkraut, 1965; Bunney and Davis, 1965), data gathered from studies of neurochemical effects of antidepressants in animals have helped form the basis of a series of theories and modifications thereof, concerning the etiology of depression and the mechanisms of action of antidepressant therapy (for review, see Stone, 1983). Currently in focus is the concept of Sulser *et al.* (1978) which, like many of its predecessors, proposes an association between central noradrenergic activity and the depressed state: specifically, the mechanism underlying successful antidepressant therapy is desensitization of the central beta-adrenergic receptor-coupled adenylate cyclase system. Evidence that 5HT receptors are also down-regulated by TCA (Peroutka and Snyder, 1980; Sugrue, 1981) and that serotonergic innervation seems to play a permissive role in beta-adrenergic receptor desensitization (Brunello *et al.*, 1982) supports the idea of an indoleamine-catecholamine link (Prange *et al.*, 1974) and has resulted in further modification of this concept (Sulser, 1984a, 1984b).

Catecholamine-indoleamine theories of the neurochemistry of depression, along with clinical findings linking thyroid states to affective disorders, have evoked considerable interest in the effects of thyroid status on monoamine turnover and central adrenergic receptors. Indeed, there have been several recent and, in some aspects, conflicting reports (Gross *et al.*, 1980a; Perumal *et al.*, 1984; Atterwill *et al.*, 1984; Schmidt and Schultz, 1985) on the effects of thyroidal condition on the status of beta-adrenergic receptors in rat cerebral cortex and other brain regions (see below). Here we present data on the effects of thyroidectomy or administration of thyroid hormones on beta-adrenergic and 5HT₂ receptor binding in rat brain and how these effects interact with receptor effects produced by chronic treatment with desmethylimipramine (DMI).

METHODS

Intact, thyroidectomized and sham-operated adult male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 125–150 g upon arrival were used in this study. Mature animals were used to avoid developmental effects of thyroid status on brain monoamine systems. Surgical thyroidectomies or sham-operations were performed by the vendor. No less than 5 days after arrival the animals were injected subcutaneously (s.c.) once daily at 1000–1200 hr for 7 or 10 consecutive days with desmethylimipramine HCl (DMI; 20 mg/kg) or vehicle (0.9% NaCl) and intraperitoneally with various doses of the free acids of T_3 (15–1000 µg/kg), T_4 (15–500 µg/kg), rT_3 (500 µg/kg) or vehicle [0.9% NaCl:methanol: NH_4OH (8 N); 396:3:1 (v/v)]. The TCA DMI was selected for use in this study because it reliably down-regulates both beta-adrenergic and 5HT₂ receptors in rat cerebral cortex (Sugrue, 1981). Throughout the treatment protocol, rats were housed two to a cage and given free access to water and laboratory chow. Animal quarters were kept at a temperature of 22–25°C, and the light-dark cycle was 12 hr.

All animals were weighed 24 hr after the last injections. They were then killed by decapitation, and blood was collected at that time for analysis of serum thyroid hormones by radioimmunoassay (T_3 , T_4 — Becton-Dickinson Immunoassay, Orangeburg, NY; rT_3 — Sero Laboratories, Braintree, MA). Brains were immediately removed and kept on ice while the corpus striatum, cerebellum and hippocampus and/or the frontal cerebral cortex were dissected out, weighed and frozen on dry ice. Brain tissues were kept frozen at –50°C until thawed for preparation of membranes for measurement of beta-adrenergic or serotonin₂ (5HT₂) receptor binding based on the procedures of Byland and Snyder (1976) or Leysen *et al.* (1982), respectively.

A crude membrane fraction was prepared from brain regions by homogenization of tissue in 19 vols 0.32 M sucrose followed by centrifugation at 3000 g for 10 min. The pellet was discarded, and the supernatant was centrifuged at 48,000 g for 10 min. The pellet was resuspended by homogenization in 40 mM Tris-HCl buffer, pH 7.4 and was recentrifuged (48,000 g for 10 min). For beta-adrenergic receptor binding a portion of the final pellet was resuspended in 40 mM

Tris-HCl (pH 7.4) buffer at a concentration corresponding to 50 mg original tissue/ml. Binding incubations were carried out in triplicate in 1 ml of medium containing 40 mM Tris-HCl pH 7.4 and [3 H]dihydroalprenolol ([3 H]DHA; 102.7 Ci/mmol) at various concentrations. The amount of tissue used per tube corresponded to 5 mg original wet weight and contained 300–400 μ g protein as determined by the method of Lowry *et al.* (1951). At the end of a 25 min incubation at 25°C, samples were filtered on glass fiber discs (25 mm diameter, 0.3 μ pore size, Gelman Inc., Ann Arbor, MI) and washed twice rapidly with 5 ml Tris buffer. Filter discs were then dried and counted in 5 ml Aquasol scintillation mixture with a Beckman LS 7000 scintillation counter at an efficiency of 38–43%. Control incubations containing unlabeled competing ligand (10^{-6} M alprenolol) were carried out simultaneously in order to determine the extent of non-specific binding (about 30%).

Membrane fractions of different regions from each animal were first assayed at a [3 H]DHA concentration of 1 nM. These data were used to compare mean receptor binding of the different treatment groups. The unused portions of membranes from animals of the same treatment groups were then pooled to obtain sufficient material for additional binding assays, in triplicate, with seven different [3 H]DHA concentrations ranging from 0.1 to 5.0 nM. These data were used for Scatchard type analysis (Scatchard, 1949). Receptor binding of [3 H]DHA in brain samples from animals in the different treatment groups was analyzed statistically with Tukey's range test (Miller, 1966) or Dunnett's test for multiple comparisons (Dunnett, 1964) after ANOVA. Significant differences were defined as *p* values of 0.05 or less.

Portions of the same membrane preparations used for studies of [3 H]DHA binding, except cerebellar membranes, were used for characterizing [3 H]ketanserin ([3 H]KET) binding: cerebellar membranes contain few 5HT₂ receptors. An assay procedure similar to the [3 H]DHA binding assay was used. Incubations were initially carried out for 15 min at 37°C in triplicate in 1 ml Tris-HCl buffers, pH 7.4, containing 1 nM [3 H]KET, and later in pooled preparations at various concentrations of the radioligand (0.5–20 nM). Non-specific binding was determined with 10^{-6} M methysergide.

All radioligands were purchased from New England Nuclear Corporation (Boston, MA); thyroid hormones were bought from either Sigma Chemical Co. (St. Louis, MO) or Hennings (Berlin, F.R.G.). DMI was a gift from the Revlon Health Care Group, Research and Development Division, Tuckahoe, NY.

RESULTS

The effects of hormone injections or thyroidectomy on body weights and serum hormone levels are presented in Table I. Levels of T₃ or T₄ in serum collected 24 hr after the last hormone injection increased with dose, as expected, and both thyroid hormones were undetectable in serum from thyroidectomized animals. Administration of higher doses of T₄ produced elevations of both T₄ and T₃ via 5'-monodeiodination of T₄, whereas the administration of T₃

TABLE I. BODY WEIGHTS AND SERUM HORMONE LEVELS AT TIME OF SACRIFICE OF RATS GIVEN VARIOUS DOSES OF THYROID HORMONES OR THYROIDECTOMIZED

Treatment	Days	Body weight (g $\pm$ SEM)	T ₃ (ng/dl $\pm$ SEM)	T ₄ (μ g/dl $\pm$ SEM)	rT ₃ (pg/ml $\pm$ SEM)
Control*		311 $\pm$ 2 (67)	56 $\pm$ 3 (67)	3.8 $\pm$ 0.4 (67)	51 $\pm$ 5 (6)
T ₄ (50 μ g/kg)	10	320 $\pm$ 7 (12)	57 $\pm$ 6 (12)	4.9 $\pm$ 0.3 (12)	—
T ₄ (100 μ g/kg)	10	290 $\pm$ 16 (6)	49 $\pm$ 5 (6)	5.7 $\pm$ 0.4 (6)	—
T ₄ (250 μ g/kg)	10	313 $\pm$ 13 (9)	83 $\pm$ 18 (9)	6.1 $\pm$ 0.6 (9)	—
T ₄ (500 μ g/kg)	7	285 $\pm$ 13 (11)	136 $\pm$ 23 (11)	8.8 $\pm$ 1.3 (11)	—
T ₃ (15 μ g/kg)	10	299 $\pm$ 10 (11)	54 $\pm$ 7 (11)	1.3 $\pm$ 0.2 (11)	—
T ₃ (100 μ g/kg)	10	286 $\pm$ 15 (6)	89 $\pm$ 11 (6)	0.7 $\pm$ 0.1 (6)	—
T ₃ (250 μ g/kg)	10	267 $\pm$ 4 (16)	212 $\pm$ 24 (15)	0.6 $\pm$ 0.1 (16)	—
T ₃ (500 μ g/kg)	7	256 $\pm$ 4 (36)	439 $\pm$ 37 (36)	<0.5 (24)	—
T ₃ (1000 μ g/kg)	7	226 $\pm$ 20 (6)	492 $\pm$ 86 (6)	<0.5 (6)	—
rT ₃ (500 μ g/kg)	7	306 $\pm$ 15 (6)	65 $\pm$ 12 (6)	4.8 $\pm$ 0.6 (6)	163 $\pm$ 44 (6)
Thyroidectomy	21–35	191 $\pm$ 7 (30)	<5 (30)	<0.5 (30)	<15 (6)

*Intact and sham-thyroidectomized control rats showed nearly identical values.
The number of animals is in parentheses.

TABLE II. THE EFFECTS OF THYROID HORMONE ADMINISTRATION OR THYROIDECTOMY ON [³H]DIHYDROALPRENOLOL (BETA-ADRENERGIC RECEPTOR) OR [³H]KETANSERIN (5HT₂ RECEPTOR) BINDING TO RAT CEREBRAL CORTEX MEMBRANE PREPARATIONS

Treatment	Days	Receptor binding (% control $\pm$ SEM)	
		Beta-adrenergic	5HT ₂
Control		100 $\pm$ 2 (67)	100 $\pm$ 2 (46)
T ₄ (50 μ g/kg)	10	104 $\pm$ 3 (12)	99 $\pm$ 3 (11)
T ₄ (100 μ g/kg)	10	106 $\pm$ 2 (6)	110 $\pm$ 4 (6)
T ₄ (250 μ g/kg)	10	111 $\pm$ 3 (9)*	111 $\pm$ 5 (9)
T ₄ (500 μ g/kg)	7	114 $\pm$ 2 (11)*	116 $\pm$ 4 (12)*
T ₃ (15 μ g/kg)	10	102 $\pm$ 4 (11)	105 $\pm$ 3 (17)
T ₃ (50 μ g/kg)	10	103 $\pm$ 9 (3)	106 $\pm$ 3 (9)
T ₃ (100 μ g/kg)	10	103 $\pm$ 2 (6)	108 $\pm$ 5 (5)
T ₃ (250 μ g/kg)	10	107 $\pm$ 2 (16)*	109 $\pm$ 4 (11)
T ₃ (500 μ g/kg)	7	115 $\pm$ 3 (36)*	111 $\pm$ 2 (20)*
T ₃ (1000 μ g/kg)	7	118 $\pm$ 4 (6)*	122 $\pm$ 3 (3)*
rT ₃ (500 μ g/kg)	7	98 $\pm$ 5 (6)	99 $\pm$ 6 (6)
Thyroidectomy	21–35	99 $\pm$ 3 (30)	98 $\pm$ 4 (6)

*Significantly different from controls, $p < 0.05$.

The number of animals is in parentheses.

elevated serum T₃ and suppressed serum T₄ levels via negative feedback. Weight gain tended to be inversely correlated with the dose of thyroid hormone; in thyroidectomized animals, weight gain was also less than in controls.

Although toxicity of thyroid hormones, alone or in combination with DMI, was not systematically studied, we noted that about 10% of animals that received the highest dose of T₃ (1 mg/kg) died before 7 consecutive days of treatment (although this dose was subsequently discontinued, the data gathered up to that time are presented). No deaths resulted from treatment with 500 μ g/kg doses of T₃ or T₄; however, deaths occurred in about 25% of animals given 500 μ g/kg T₃ in combination with 20 mg/kg DMI. No deaths resulted from the combination of 500 μ g/kg T₄ and 20 mg/kg DMI.

The effects of various doses of thyroid hormones or thyroidectomy on receptor binding of [³H]DHA and [³H]KET in rat cerebral cortex are summarized in Table II. Administration of either T₄ or T₃ for 7 or 10 days produced a dose-dependent increase in beta-adrenergic and 5HT₂ receptor binding as compared to vehicle injected controls. No change in binding to either receptor was detected in cortices of animals injected for 7 days with 500 μ g/kg rT₃ or thyroidectomized 21–42 days before killing, compared to intact or sham-operated controls.

Scatchard plots of binding data for pooled membrane preparations from animals of various treatment groups were straight lines. Maximum binding ($B_{\max}$) and receptor affinity for [³H]DHA or [³H]KET (K_D) of pooled membrane preparations derived from analysis of these data are presented in Table III. They indicate that the dose-related increased binding of [³H]DHA or [³H]KET induced by T₃ and T₄ (Table I) probably resulted from an increase in receptor number. We also observed a small but consistent decrease in the affinity for [³H]KET, with increasing dosage of T₃ and T₄. Perumal *et al.* (1984), using an assay procedure like the one used here, found that neither T₄ nor T₃ interfered with the specific binding of [³H]DHA.

TABLE III. SCATCHARD ANALYSIS OF BINDING DATA FROM POOLED CORTICAL MEMBRANES. MAXIMUM BINDING ($B_{\max}$) AND AFFINITY (K_D) WERE DERIVED FOR [^3H]DHA (BETA-ADRENERGIC RECEPTORS) AND [^3H]KET (5HT₂ RECEPTORS) USING VARIOUS CONCENTRATIONS OF RADIOLIGAND

Treatment	Days	[^3H]DHA binding			[^3H]KET binding		
		$B_{\max}$ (fmol/mg protein ± SEM)	K_D (nM ± SEM)	r	$B_{\max}$ (fmol/mg protein ± SEM)	K_D (nM ± SEM)	r
Control		72 ± 3	0.64 ± 0.05	0.99	165 ± 9	0.49 ± 0.11	0.99
T ₄ (50 µg/kg)	10	78 ± 3	0.67 ± 0.03	0.99	168 ± 1	0.40 ± 0.01	0.99
T ₄ (100 µg/kg)	10	77 ± 4	0.79 ± 0.21	0.96	165 ± 2	0.32 ± 0.02	0.91
T ₄ (250 µg/kg)	10	87 ± 6	1.10 ± 0.15	0.94	184 ± 7	0.58 ± 0.08	0.96
T ₄ (500 µg/kg)	7	87 ± 5	1.10 ± 0.13	0.99	186 ± 8	0.53 ± 0.07	0.98
T ₃ (15 µg/kg)	10	71 ± 2	0.56 ± 0.02	0.99	—	—	—
T ₃ (50 µg/kg)	10	79 ± 2	0.66 ± 0.02	0.99	173 ± 2	0.32 ± 0.01	0.98
T ₃ (100 µg/kg)	10	80 ± 4	0.68 ± 0.20	0.95	171 ± 3	0.36 ± 0.02	0.97
T ₃ (250 µg/kg)	10	79 ± 3	0.87 ± 0.07	0.93	176 ± 5	0.52 ± 0.04	0.90
T ₃ (500 µg/kg)	7	87 ± 5	1.10 ± 0.33	0.96	194 ± 6	0.43 ± 0.04	0.98
Thyroidectomy	21–35	70 ± 5	0.61 ± 0.06	0.97	167 ± 6	0.51 ± 0.09	0.97

TABLE IV. THE EFFECTS OF THYROID HORMONES OR THYROIDECTOMY ON [^3H]DIHYDROALPRENOLOL (BETA-ADRENERGIC RECEPTORS) AND [^3H]KETANSERIN (5HT₂ RECEPTORS) BINDING IN MEMBRANE PREPARATION OF RAT CEREBELLUM, STRIATUM AND HIPPOCAMPUS

Treatment	Days	Beta-adrenergic receptor binding (% control ± SEM)			5HT ₂ receptor binding (% control ± SEM)	
		Cerebellum	Striatum	Hippocampus	Striatum	Hippocampus
Control		100 ± 5 (23)	100 ± 3 (24)	100 ± 3 (12)	100 ± 1 (7)	100 ± 4 (15)
T ₄ (50 µg/kg)	10	95 ± 11 (6)	97 ± 4 (6)	105 ± 2 (16)	98 ± 7 (6)	96 ± 3 (6)
T ₄ (100 µg/kg)	10	97 ± 4 (6)	96 ± 3 (6)	102 ± 3 (5)	111 ± 10 (5)	99 ± 4 (6)
T ₄ (250 µg/kg)	10	108 ± 4 (9)	107 ± 4 (10)	113 ± 5 (10)	114 ± 4 (10)*	114 ± 3 (10)*
T ₄ (500 µg/kg)	7	109 ± 7 (4)	107 ± 5 (6)	114 ± 2 (6)*	114 ± 3 (6)*	116 ± 7 (6)
T ₃ (15 µg/kg)	10	102 ± 11 (6)	98 ± 4 (5)	109 ± 6 (6)	97 ± 10 (6)	91 ± 8 (6)
T ₃ (100 µg/kg)	10	110 ± 10 (6)	102 ± 5 (6)	110 ± 7 (6)	105 ± 9 (6)	90 ± 9 (6)
T ₃ (250 µg/kg)	10	115 ± 10 (6)	106 ± 4 (11)	115 ± 3 (12)*	106 ± 4 (12)	100 ± 5 (12)
T ₃ (500 µg/kg)	7	—	105 ± 5 (6)	—	105 ± 4 (6)	—
Thyroidectomy	35	84 ± 3 (6)*	99 ± 5 (67)	—	86 ± 6 (6)	—

*Significantly different from controls, $p < 0.05$.

The number of animals is in parentheses.

We observed that neither 10^{-5} M T₃ nor T₄ had an effect on the binding of [^3H]DHA or [^3H]KET.

The effects of thyroid hormones on beta-adrenergic and 5HT₂ receptor binding in the striatum, cerebellum (beta-adrenergic receptor binding only) and hippocampus are described in Table IV. Administration of thyroid hormones significantly increased binding to both types of receptors only in the hippocampus, whereas hypothyroidism only reduced beta-adrenergic receptors in the cerebellum. Scatchard analysis of [^3H]DHA binding in pooled hippocampal membrane preparations indicated that the increase in binding in animals that received 250

TABLE V. THE EFFECTS OF PHARMACOLOGICAL AGENTS WITH AND WITHOUT THYROID HORMONES OR THYROIDECTOMY ON [3 H]DIHYDROALPRENOLOL (BETA-ADRENERGIC RECEPTORS) AND [3 H]KETANSERIN (5HT $_2$ RECEPTORS) BINDING IN MEMBRANE PREPARATIONS OF RAT CEREBRAL CORTEX

Treatment	Days	Receptor binding (% control $\pm$ SEM)	
		Beta-adrenergic	5HT $_2$
Control		100 $\pm$ 2 (67)*	100 $\pm$ 2 (46)*
Reserpine (0.5 mg/kg)	7	144 $\pm$ 5 (8)*†	113 $\pm$ 4 (6)*
DMI (10 mg/kg)	10	83 $\pm$ 6 (6)†	95 $\pm$ 5 (6)*
DMI (20 mg/kg)	7	71 $\pm$ 3 (24)†	78 $\pm$ 3 (24)†
DMI (10 mg/kg) + T $_3$ (15 μ g/kg)	10	83 $\pm$ 5 (6)†	102 $\pm$ 3 (6)*
DMI (20 mg/kg) + T $_3$ (500 μ g/kg)	7	79 $\pm$ 3 (14)†	79 $\pm$ 3 (14)†
DMI (20 mg/kg) + T $_4$ (500 μ g/kg)	7	85 $\pm$ 4 (5)*†	81 $\pm$ 4 (5)†
Thyroidectomy + DMI (20 mg/kg)	7	75 $\pm$ 5 (6)†	—

*Significantly different from DMI (20 mg/kg), $p < 0.05$.

†Significantly different from controls, $p < 0.05$.

The number of animals is in parentheses.

μ g/kg T $_3$ ($B_{\max} = 103 \pm 11$ fmol/mg protein; $K_D = 2.2 \pm 0.45$ nM) compared to controls ($B_{\max} = 88 \pm 5$ fmol/mg protein; $K_D = 1.5 \pm 0.08$ nM) was more likely the result of an increased number of receptors rather than increased receptor affinity. The reduction in [3 H]DHA binding in cerebellum ($B_{\max} = 67 \pm 7$ fmol/mg protein; $K_D = 1.6 \pm 0.3$ nM) of thyroidectomized rats compared with controls ($B_{\max} = 75 \pm 7$ fmol/mg protein; $K_D = 2.1 \pm 0.2$ nM) probably resulted from a decrease in binding sites rather than a reduction in affinity, as did the reduction in [3 H]KET binding in striatum of thyroidectomized rats ($B_{\max} = 85 \pm 2$ fmol/mg protein; $K_D = 1.0 \pm 0.3$ nM) compared to control rats ($B_{\max} = 94 \pm 5$ fmol/mg protein; $K_D = 0.9 \pm 0.1$ nM).

The effects on receptor binding of pharmacological agents with and without thyroid hormones or thyroidectomy are presented in Table V. The tricyclic antidepressant DMI produced a down-regulation of both beta-adrenergic and 5HT $_2$ receptors. As expected, an opposite effect was produced by 7 days of reserpine treatment. The down-regulation of beta-adrenergic receptors produced by DMI was significantly attenuated by 500 μ g T $_4$; the two treatments produced opposite effects that were offsetting. However, the significant but slightly less potent down-regulation of 5HT $_2$ receptor binding produced by DMI was apparently not antagonized by concurrent administration of either T $_3$ or T $_4$. The effect of DMI in thyroidectomized rats was not different from that in sham-operated or intact controls.

DISCUSSION

Our study demonstrates that peripheral administration of thyroid hormones produces specific dose-dependent changes in two populations of cerebral cortical receptors in neurotransmitter pathways affected by antidepressant therapy and thought to be involved in the etiology of depression. Similar studies of thyroidally induced changes on beta-adrenergic receptors have been conducted by other investigators, often using different strains of rats, different manipulations of the thyroid axis and different methods of measuring receptor binding. Understandably, the effects of thyroid status on this receptor type are not completely clear, and there are areas of seeming contradiction.

Our data indicate that chronic thyroid hormone administration produces a dose-related increase in [3 H]DHA binding to cortical beta-adrenergic receptors. We observed that significant differences from vehicle-injected controls occurred consistently only in animals that received a relatively large dose of T_3 or T_4 ($> 100 \mu\text{g/kg}$). In general, this finding is consistent with that of Perumal *et al.* (1984), who observed a significant up-regulation of cortical beta-adrenergic receptors after dosing rats with $375 \mu\text{g/kg}$ T_4 for 7 consecutive days, and with the work of Atterwill *et al.* (1984), who reported no significant change in this receptor after giving Porton strain rats $100 \mu\text{g/kg}$ T_3 for 14 days. Our findings are, however, apparently discordant with those of Schmidt and Schultz (1985), who reported that small doses of T_3 ($15 \mu\text{g/kg}$) or T_4 ($50 \mu\text{g/kg}$) down-regulated the noradrenergic cyclic AMP generating system and the number of beta-adrenergic receptors in the cerebral cortex of Sprague-Dawley and Long-Evans, but not of Wistar and F-344, rats.

We also observed that the affinity of beta-adrenergic receptors for [3 H]DHA decreased with increasing doses of T_3 and T_4 . Although we were unable to provide an adequate explanation for this binding decrease, it does not appear to be an artifact of interference by T_3 or T_4 in the binding assay, and the modest increases in receptor number suggest that steric hindrance is not involved. It is more likely that thyroid hormones produce some membrane change(s) which preferentially affect the affinity of the beta-receptor.

We did not find any significant change in rat cortical beta-adrenergic receptor binding 28–42 days after thyroidectomy. This is also in agreement with the finding of Atterwill *et al.* (1984) in rats made hypothyroid by treatment with 6-propyl-2-thiouracil (PTU) for 2 weeks, but not in agreement with Gross *et al.* (1980a), who reported a significant reduction of cortical beta-adrenergic receptors in Wistar rats after 6 weeks of PTU treatment. Atterwill *et al.* (1984) attributed this difference to morphological changes in the brains of chronically hypothyroid rats.

Although most authors have focused their studies of drug- or hormone-induced adaptation of beta-adrenergic receptors on the cerebral cortex, others have noted changes in other brain regions. Like Perumal *et al.* (1984) and Atterwill *et al.* (1984), we observed some regional differences in the effects of thyroid status on beta-adrenergic receptors. We observed no significant effects on beta-adrenergic receptor binding in striatal tissue after administration of relatively large doses of thyroid hormones or after thyroidectomy, but we did find a 16% decrease in binding in cerebellar membranes of thyroidectomized rats. Large doses of T_3 or T_4 increased [3 H]DHA binding in the hippocampus. Binding of [3 H]KET in the striatum was increased by T_4 and decreased by thyroidectomy. A similar increase in [3 H]KET binding was observed in hippocampal membranes of hyperthyroid rats; tissue from this region from thyroidectomized animals was not studied.

We also observed the previously described (Banerjee *et al.*, 1977) down-regulation of beta-adrenergic receptors after chronic administration of DMI. As expected on the basis of our results with thyroid hormone administration alone, we found that concurrent administration of only large doses of thyroid hormones had a significant antagonistic effect on the down-regulation produced by the drug. In a study of the interactions of DMI and T_3 , Atterwill *et al.* (1986) noted that a single dose of T_3 ($100 \mu\text{g/kg}$) on day 1 slightly decelerated the rate of beta-adrenergic receptor down-regulation induced by daily injections of DMI (5 mg/kg) for 14 days. It is interesting that DMI was effective in down-regulating beta-adrenergic receptors of thyroidectomized rats; it has been suggested, though only by anecdotal evidence, that TCAs are not as effective in hypothyroid patients (Avni *et al.*, 1967). If down-regulation occurs in

hypothyroid depressed patients, even as these patients show a poor or null antidepressant response, the down-regulation cannot be a sufficient explanation for the usual therapeutic action of TCAs.

The dose-related increase in 5HT₂ receptor binding in the cortex, striatum and hippocampus produced by peripheral administration of thyroid hormones has not been reported previously. Although in most cases the thyroidally induced changes in the 5HT₂ and beta-adrenergic receptors were in the same direction and of similar degree, thyroidectomy reduced the number of 5HT₂ receptors in the striatum but produced no effect on beta-adrenergic receptor binding. Furthermore, we saw no significant antagonism of the DMI-induced 5HT₂ receptor down-regulation in the cerebral cortex by either T₃ or T₄.

Our general finding that significant changes in either beta-adrenergic or 5HT₂ receptor binding are induced only by large doses of thyroid hormones is consistent with evidence, also in rats, that the thyroid status of the brain remains relatively stable in the face of marked changes in peripheral thyroid hormone levels (Dratman *et al.*, 1983). Of course, this does not preclude the possibility that small changes may have substantial functional effects. The directions of change in beta-adrenergic receptors which we report here are generally consistent with our previous work (Lipton *et al.*, 1968; Prange *et al.*, 1970) and the work of others (Parker, 1972), indicating that catecholamine synthesis and turnover in brain are reduced in hyperthyroidism and increased in hypothyroidism. However, opposite effects also have been reported (Engstrom *et al.*, 1974; Jacoby *et al.*, 1975; Strombom *et al.*, 1977), and it is not known whether the effects of thyroid hormones on this receptor or the 5HT₂ receptor population result from an adaptation to changes in synaptic level of endogenous ligands, stimulation of protein synthesis or other mechanisms.

Although the major emphasis of the work of Schmidt and Schultz (1985) is on cortical cyclic AMP generating systems, and not the beta-adrenoceptor *per se*, their finding that T₃ or T₄ down-regulates cortical beta-receptors in some strains of rats seems discordant with the present report and that of Perumal *et al.* (1984), as well as with earlier studies by Frazer *et al.* (1974) and Gross *et al.* (1980b) on the effects of thyroid status on the cortical adenylate cyclase system. Nevertheless, in the light of theories that advocate down-regulation of beta-adrenergic receptor-coupled adenylate cyclase systems as the mechanism of action of antidepressant drugs, their findings are consistent with the clinical observation that small doses of thyroid hormones can potentiate the action of antidepressant drugs. Although it is speculative to infer from studies of rats what occurs or does not occur in man, our data suggest that mechanisms other than changes in postsynaptic noradrenergic or serotonergic receptors may well be involved in the potentiation of antidepressants by small amounts of thyroid hormones, and that behavioral and affective changes associated with small disturbances in peripheral levels of thyroid hormones are probably not produced by or reflected in conspicuous changes in the binding of beta-adrenergic or 5HT₂ receptors.

A problem inherent in studying brain effects of thyroid states as defined by peripheral criteria is that, in rats at least, thyroid states of the brain and periphery may be dissociated. For example, it has been shown that DMI reduces the levels of T₄ in synaptosomes without altering plasma T₄ levels (Dratman and Crutchfield, 1979), while in starvation brain T₃ remains relatively unchanged as plasma T₃ levels decrease sharply (Larsen *et al.*, 1981). Elucidating the neuronal effects of different thyroid states and their relationships to behavior and affect remain an important challenge.

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