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GABA UPTAKE IS INHIBITED BY THYROID HORMONES: IMPLICATIONS FOR DEPRESSION

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SUMMARY

Studies of the effects of thyroid hormones on the uptake of neurotransmitters by homogenates of rat cerebral cortex have revealed a significant competitive inhibition of neuronal uptake of [^{3}H]GABA by thyroid hormones ($T_3 > T_4 > rT_3$). The IC_{50} for inhibition of GABA uptake by T_3 was estimated at 4 μM and that of T_4 at 11 μm. GABA uptake in homogenates of cerebral cortex from hypothyroid rats was significantly enhanced over that of controls; however, uptake in tissues from hyperthyroid rats was not significantly diminished.

INTRODUCTION

THE INFLUENCE of hormones on behavior has long been recognized. Perhaps most notable are the effects of thyroidal condition on affective state. Hypothyroidism is commonly associated with a depressed mood and a deficit in recent memory; these are usually alleviated if euthyroidism is reinstated (Whybrow *et al.*, 1969). Our clinical group as well as others have given the thyroid hormone L-3,3′,5-triiodothyronine (T_3) with a tricyclic antidepressant (TCA) to patients suffering from depression and have noted an enhanced clinical response (Wilson *et al.*, 1970; Earle, 1970; Coppen *et al.*, 1972; Wheatly, 1972; Hatotani *et al.*, 1974; Ogura *et al.*, 1974; Goodwin *et al.*, 1982). We now have directed laboratory studies toward the elucidation of this phenomenon.

It has been shown that thyroxine (T_4) is taken up into the synaptosomal fraction of brain neurons and there converted to its more potent metabolite, T_3 (Dratman & Crutchfield, 1978). T_3 directly stimulates transport processes in neurons and nerve endings (Iqbal *et al.*, 1984). These findings support the concept of a functional role of thyroid hormones at the synapse. Efforts toward investigation of the synaptic effects of thyroid hormones have been focused on the noradrenergic system, which seems a likely site for a thyroidal–TCA interaction. However, we have now broadened our investigation to include the effects of thyroid hormones on the synaptic components of other neurotransmitter systems, including the gamma-aminobutyric acid (GABA) system. Our interest in the GABA system derives from three observations: low levels of GABA in cerebrospinal fluid in depression (Gold *et al.*, 1980; Gerner & Hare, 1981), success of GABA mimetics in treating this disorder (Lloyd *et al.*, 1983), and the effects of chronic

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treatment with TCA on [^{3}H]GABA binding to receptors in brain tissue of rats (Lloyd & Pilc, 1984). We report herein a specific inhibition of GABA uptake by thyroid hormones, and increased GABA uptake in hypothyroidism.

METHODS

Adult male Sprague – Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) weighing 125 – 200 g upon arrival were used in this study. In some animals hypothyroidism was produced by surgical thyroidectomy (performed by the vendor), and hyperthyroidism was produced in sham-operated rats by once daily intraperitoneal injection of 500 μg/kg T_3 for 7 days. Thyroidectomized and control rats were injected on the same schedule with the T_3 vehicles [0.9% NaCl; methanol; NH_4OH; 396:3:1 (v/v)]. Rats were housed two to a cage and given free access to water and laboratory chow. Animal quarters were kept at a temp of 22 – 25°C, and the light:dark cycle was 12:12 hr.

Animals were decapitated after various treatments, and brains were immediately removed and kept on ice while the frontal cortex was dissected out and weighed. Cortical tissue was homogenized in 19 vol of 0.32 M sucrose, and the resulting homogenate was further diluted with 0.32 M sucrose, to a 1% (w/v) tissue concentration, so that uptake of each neurotransmitter was proportional to tissue content. Trunk blood was collected into glass tubes at the time of killing and allowed to clot at 4°C. Serum concentrations of T_3 were determined by commercial radioimmunoassay (Becton-Dickinson) Co., Orangeburg, NY).

The standard incubation medium for the *in vitro* uptake assay consisted of Krebs – Ringer buffer containing 150 mM NaCl, 5 mM KCl, 1.5 mM $CaCl_2$, 1.4 mM $MgSO_4$, 1.2 mM ascorbic acid, 25 mM glucose, 0.1 mM aminooxyacetic acid (an inhibitor of GABA transaminase), and 0.1 mM pargyline (an inhibitor of monoamine oxidase) in 40 mM Tris – HCl, at pH 7.4 (Kuhar, 1973). The uptake assay was initiated by adding 0.1 ml tritiated neurotransmitter (New England Nuclear, Boston, MA) dissolved in medium buffer to a tube containing 0.8 ml medium and 0.1 ml dilute tissue homogenate. The final concentration of each compound was 10^{-8}M: [^{3}H]dopamine ([^{3}H]DA), 31.6 Ci/mmol; [^{3}H]GABA, 34.9 Ci/mmol; serotonin ([^{3}H]5HT), 27.3 Ci/mmol; choline ([^{3}H]Ch), 180 Ci/mmol; and d-aspartate ([^{3}H]Asp), 14 Ci/mmol. Thyroid hormones were dissolved in 3:1 (v/v) methanol:NH_4OH and diluted to the appropriate concentration with medium buffer before addition to the incubation mixture. Other substances, including L-diaminobutyric acid (DABA), beta-alanine and desmethylimipramine (DMI), were initially dissolved in medium buffer alone.

Incubation was at 37°C with continuous shaking for 5 min. Identical mixtures were incubated at 0°C for 5 min to control for non-energy dependent neurotransmitter binding. Triplicate samples were used for both 37 and 0°C assays. A short incubation and the addition of enzyme inhibitors to the medium was used to minimize any metabolic conversion of the tritiated compounds.

The incubation was terminated by replacing the samples on ice followed by immediate vacuum filtration through a Millipore manifold fitted with Gelman Type A/E fiber filters (pore size 0.3 μm). The filters were washed three times with 5 ml of ice cold 40 mM Tris, pH 7.4 to remove any non-transported isotope, transferred to scintillation vials with 5 ml

Aquasol (New England Nuclear), and counted in a Beckman LS 7000 liquid scintillation counter.

Data were analyzed using Dunnett's test for multiple comparisons (Dunnett, 1964), after ANOVA. Significance was defined by p values of 0.05 or less.

RESULTS

The effects of thyroid hormones on the uptake of neurotransmitters by homogenates from cerebral cortex of euthroid rats are summarized in Table I. The thyroid hormone vehicle had no effect on the uptake of any neurotransmitter tested. The uptake of [^{3}H]GABA was significantly inhibited by T_3 at concentrations of 10^{-5} and 10^{-6}M. There was no significant effect of 10^{-5}M T_3 on the uptake of [^{3}H]DA, [^{3}H]ASP, or [^{3}H]CH, but there was a small, statistically significant effect on ^{3}H-5-HT uptake.

The effect of T_4 on [^{3}H]GABA uptake was weaker than that of T_3 at all concentrations of thyroid hormones tested. T_4 had a smaller inhibiting effect on [^{3}H]5-HT uptake than did T_3. Like T_3, T_4 did not significantly affect the uptake of other neurotransmitters.

Further experiments were performed to characterize the inhibitory effects of T_3 and T_4 on GABA uptake. We found that $70 \pm 2\%$ of the uptake of [^{3}H]GABA in the homogenates could be blocked by 10^{-4}M DABA, an inhibitor of neuronal GABA uptake (Schon & Kelly, 1974), but the same concentration of beta-alanine (an inhibitor of GABA uptake by glial cells) (Schon & Kelly, 1974) inhibited only $9 \pm 1\%$ of [^{3}H]GABA uptake. This indicated that the uptake measured was characteristic of the neuronal and not the glial uptake system. From measurements of [^{3}H]GABA uptake in the presence of different concentrations of these hormones, the IC_{50} for T_3 was estimated at 4 μM and that of T_4 at 11 μm (Fig. 1). Both T_3 and T_4 were more effective than DABA as inhibitors of GABA uptake; DABA has an IC_{50} of about 50 μM (Schon & Kelly, 1974). Analysis of Lineweaver – Burk plots of [^{3}H]GABA uptake at six different concentrations (from 5×10^{-9} to 5×10^{-8}M) in the presence of 0, 10^{-6}M, or 5×10^{-6}M T_3 and 0, 5×10^{-6}M, or 3×10^{-5}M T_4 indicated that both T_3 and T_4 act as competitive inhibitors of GABA uptake (Fig. 2). Reverse T_3 (L-3,3′,5′-triiodothyronine), a metabolically inactive thyroid hormone, did not inhibit the uptake of [^{3}H]GABA at concentrations of 10^{-5} or 10^{-6}M.

We also measured in homogenates the effects of the TCA DMI (5×10^{-6}M) on the uptake of the five transmitters named above and the uptake of [^{125}I]T_4 (10^{-8}M) or [^{125}I]T_3 (10^{-8}M), using the techniques already described. This experiment was performed mainly to establish the validity of our assay system. DMI, as expected, inhibited 35% of the uptake of [^{3}H]DA and 65% of the uptake of [^{3}H]5-HT but did not inhibit the uptake of [^{3}H]GABA, [^{3}H]ASP, or [^{3}H]CH. It inhibited about 25% of the uptake of T_4, which is concordant with an earlier report by Dratman & Crutchfield (1979), but it did not inhibit the uptake of T_3.

We next compared the uptake of [^{3}H]GABA in homogenates from cerebral cortices of groups of hyperthyroid and hypothyroid animals (Table II). Two groups of animals were found to be hyperthyroid as judged by inhibition of weight gain and elevated serum levels of T_3 at the time of killing, the animals which received sham-operations and T_3, and the animals that received thyroidectomy and T_3. In neither group was the uptake of [^{3}H]GABA by homogenates significantly different from that in controls. One group of

Table 1. The effects of T_3 and T_4 on the *in vitro* uptake of γ-aminobutryic acid (GABA), dopamine (DA), choline (CH), serotonin (5-HT), and aspartate (ASP) in homogenates of rat cerebral cortex. Each value represents the mean of at least six determinations

Inhibitor (concentration)	Neurotransmitter uptake (% control ± SEM)				
	[^{3}H]GABA	[^{3}H]DA	[^{3}H]CH	[^{3}H]5HT	[^{3}H]ASP
T_3 (10^{-5}M)	*31.7 ± 0.9	98.9 ± 4.2	104.4 ± 6.5	*80.6 ± 2.7	108.4 ± 3.1
(10^{-6}M)	*77.5 ± 3.0	94.2 ± 3.4	97.5 ± 3.4	*89.3 ± 1.9	108.9 ± 3.4
T_4 (10^{-5}M)	*63.9 ± 4.4	99.7 ± 5.0	104.1 ± 3.0	*82.6 ± 1.4	100.9 ± 3.5
(10^{-6}M)	*83.9 ± 3.9	93.6 ± 3.9	91.4 ± 5.4	93.5 ± 3.2	103.0 ± 3.2

*$p < 0.05$ compared to controls.

animals was found to be hypothyroid as judged by inhibition of weight gain and undetectable serum levels of T_3. The uptake of [^{3}H]GABA in homogenates derived from these animals was significantly enhanced.

DISCUSSION

We have described for the first time a specific inhibition by T_3 and T_4 of the high affinity neuronal uptake of [^{3}H]GABA in homogenates of rat cerebral cortex. However, at this time, the physiological significance of this affect is unknown. Although the whole brain concentrations of T_4 and T_3 are in the low nanomolar range (Dratman *et al.*, 1983), and therefore nearly three orders of magnitude below the IC_{50}s determined for T_3 and T_4, it is not inconceivable that at the synaptic membrane they may be high enough to elicit effects such as those which we have described. Interestingly, the *in vitro* inhibition of [^{3}H]GABA by T_3 or T_4 is physiologically concordant with the observed increase in [^{3}H]GABA uptake in cortical homogenates of hypothyroid rats; however, there is nothing

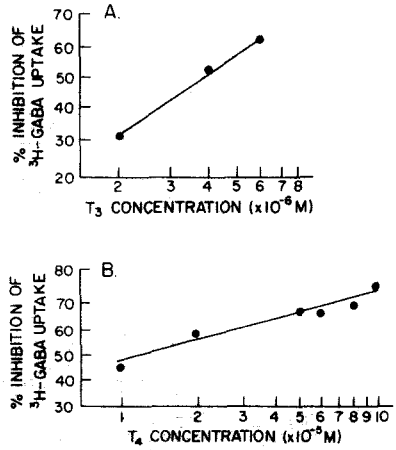

FIG. 1. [^{3}H]GABA (10^{-6}M) uptake in fresh homogenates of cerebral cortex was measured in the presence of different concentrations of T_3 (A) and T_4 (B). The IC_{50} for T_3 was estimated at 4 μM and that of T_4 at 11 μM.

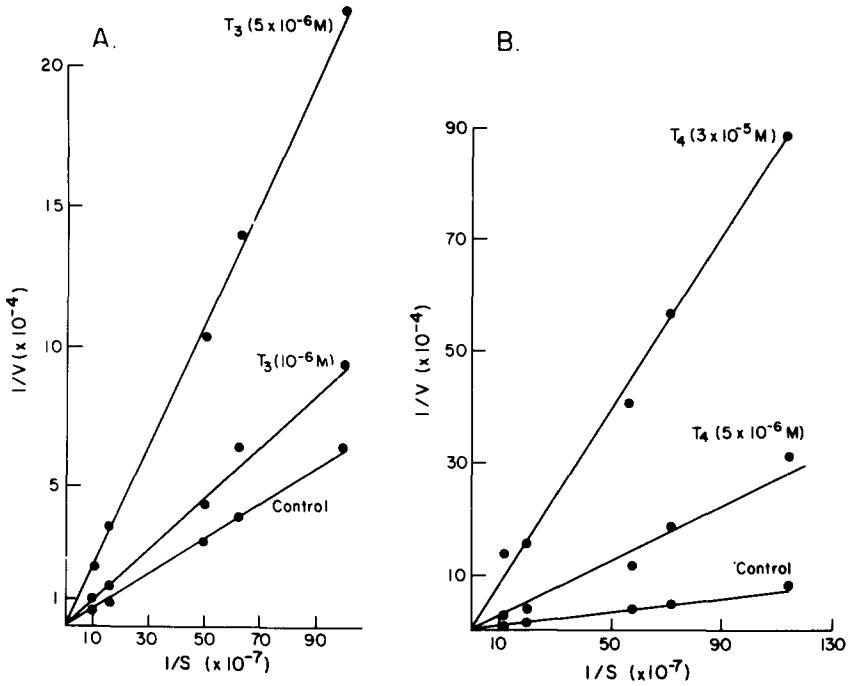

FIG. 2. Lineweaver – Burk plots of [^{3}H]GABA uptake in fresh homogenates of cerebral cortex measured at five different concentrations of [^{3}H]GABA (from 5 × 10^{-9} to 5 × 10^{-8}M) in the presence of 0, 10^{-6}M, or 5 × 10^{-6}M T_3 (A), or 0, 5 × 10^{-6}M, or 3 × 10^{-5}M T_4 (B). Both T_3 and T_4 act as competitive inhibitors of GABA uptake.

Table II. Uptake of [^{3}H]GABA in fresh homogenates from cerebral cortices of thyroidectomized or sham-operated rats injected once daily with 500 μg/kg T_3, or vehicle, for seven days. Operations were performed four weeks prior to killing

Treatment	N	Body weights (g)	Serum T_3 levels (ng/dl)	Uptake of [^{3}H]GABA (% control ± SEM)
Sham operation (Control)	6	241 ± 3.2	53 ± 4	100.0 ± 3
Thyroidectomy	6	162 ± 6.2*	< 5 *	119.0 ± 5*
Sham operation + 500 μg/kg T_3	6	190 ± 7.3*	225 ± 22*	94.0 ± 3
Thyroidectomy + 500 μg/kg T_3	6	141 ± 6.4*	740 ± 39*	99.0 ± 5

*$p < 0.05$ compared to control.

suggesting a common mechanism of action. The inhibition of [^{3}H]GABA uptake by added T_3 or T_4 is almost certainly a direct effect on GABA transport mediated at the level of the neuronal membrane, whereas the increased levels of [^{3}H]GABA uptake found in tissues from hypothyroid rats presumably result from the lack of interaction of thyroid hormones at nuclear receptors (Sterling, 1979).

Behaviorally and physiologically, T_3 and T_4 produce general excitatory effects, some of which are mediated through activation of adrenergic systems (Dratman, 1974), whereas GABA generally produces inhibition of target neurons (Roberts, 1984). The order of metabolic potency of the thyroid hormones ($T_3 > T_4 > rT_3$) is the same as their order of potency in inhibiting the uptake of GABA. Our findings that T_3 and T_4 inhibit GABA uptake might represent a mechanism whereby thyroid-induced excitation would be limited: increased levels of T_3 in brain synapses might inhibit GABA uptake and thereby facilitate GABAergic neurotransmission. Therefore, thyroid state may affect various behaviors through its effect on GABA systems and other neurotransmitter systems that interact with GABA systems, such as the DA system (Garbutt & van Kammen, 1983). Reports that hyperthyroidism potentiates, and hypothyroidism reduces, the cataleptic effects of the DA antagonist haloperidol in rats (Atterwill, 1981; Crocker & Overstreet, 1984), and that hyperthyroidism decreases the stereotypic behavior of the DA agonist apomorphine in mice (Strombon *et al.*, 1977), are consistent with an inhibitory role of thyroid hormones mediated through an inhibitory GABA system.

Regarding the effects of thyroid state on depression, much work has been done on aromatic amine neurotransmitter systems, especially in the areas of neurotransmitter synthesis and turnover and changes in pre- and post-synaptic receptor populations (Strombon *et al.*, 1977; Prange *et al.*, 1970; Engstrom *et al.*, 1974; Sugrue, 1981; Perumal *et al.*, 1984; Gross *et al.*, 1980, 1981; it now appears possible that, in addition, thyroid hormones may modify depression by inhibiting GABA reuptake inactivation and prolonging its action in the synapse.

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