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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 [3H]GABA by thyroid hormones (T₃ > T₂ > rT₄). The IC₅₀ for inhibition of GABA uptake by T₃ was estimated at 4 µM and that of T₂ 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 thyroid 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 (T₃) 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 (T₄) is taken up into the synaptosomal fraction of brain neurons and there converted to its more potent metabolite, T₃ (Dratman & Crutchfield, 1978). T₃ 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, for 7 days. Thyroidectomized and control rats were injected on the same schedule with the T, vehicles 0.9% NaCl; methanol; NH₄OH; 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, 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 CaCl₂, 1.4 mM MgSO₄, 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 $10^{-4}$M: $^3$H-dopamine ($^3$HIDA), 31.6 Ci/mmol; $^3$H-GABA, 34.9 Ci/mmol; serotonin ($^3$H5HT), 27.3 Ci/mmol; choline ($^3$HCh), 180 Ci/mmol; and d-aspartate ($^3$HAsp), 14 Ci/mmol. Thyroid hormones were dissolved in 3:1 (v/v) methanol:NH₄OH 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 desmethyldimipramine (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 \(^{1^3}H\)GABA was significantly inhibited by T\(_3\) at concentrations of \(10^{-4}\) and \(10^{-5}\)M. There was no significant effect of \(10^{-5}\)M T\(_3\) on the uptake of \(^{1^3}H\)DA, \(^{1^3}H\)ASP, or \(^{1^3}H\)CH, but there was a small, statistically significant effect on \(^3^H\)-5-HT uptake.

The effect of T\(_3\) on \(^{1^3}H\)GABA uptake was weaker than that of T\(_4\), at all concentrations of thyroid hormones tested. T\(_4\) had a smaller inhibiting effect on \(I^3H\) 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 ± 2% of the uptake of \(^{1^3}H\)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 ± 1% of \(^{1^3}H\)GABA uptake. This indicated that the uptake measured was characteristic of the neuronal and not the glial uptake system. From measurements of \(^{1^3}H\)GABA uptake in the presence of different concentrations of these hormones, the IC\(_{50}\) for T\(_3\) was estimated at 4 \(\mu\)M and that of T\(_4\) at 11 \(\mu\)M (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 \(\mu\)M (Schon & Kelly, 1974). Analysis of Lineweaver–Burk plots of \(^{1^3}H\)GABA uptake at six different concentrations (from \(5 \times 10^{-5}\) to \(5 \times 10^{-4}\)M) in the presence of 0, \(10^{-6}\)M, or \(5 \times 10^{-6}\)M T\(_3\), and 0, \(5 \times 10^{-6}\)M, or \(3 \times 10^{-6}\)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 \(^{1^3}H\)GABA at concentrations of \(10^{-5}\) or \(10^{-4}\)M.

We also measured in homogenates the effects of the TCA DMI (5 \(\times\) \(10^{-5}\)M) on the uptake of the five transmitters named above and the uptake of \(^{12^5}\)IT\(_3\) (10\(^{-4}\)M) or \(^{12^5}\)IT\(_4\), (10\(^{-4}\)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 \(^{1^3}H\)DA and 65% of the uptake of \(^{1^3}H\)5-HT but did not inhibit the uptake of \(^{1^3}H\)GABA, \(^{1^3}H\)ASP, or \(^{1^3}H\)CH. It inhibited about 25% of the uptake of T\(_3\), which is concordant with an earlier report by Dratman & Crutchfield (1979), but it did not inhibit the uptake of T\(_4\).

We next compared the uptake of \(^{1^3}H\)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 \(^{1^3}H\)GABA by homogenates significantly different from that in controls. One group of
Table I. The effects of T<sub>3</sub> and T<sub>4</sub> on the in vitro uptake of γ-aminobutyric 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.

<table>
<thead>
<tr>
<th>Inhibitor (concentration)</th>
<th>&lt;sup&gt;1&lt;/sup&gt;HIGABA (% control ± SEM)</th>
<th>&lt;sup&gt;1&lt;/sup&gt;HIDA</th>
<th>&lt;sup&gt;1&lt;/sup&gt;HICH</th>
<th>&lt;sup&gt;1&lt;/sup&gt;H5HT</th>
<th>&lt;sup&gt;1&lt;/sup&gt;HASP</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; (10&lt;sup&gt;-3&lt;/sup&gt;M)</td>
<td>*31.7 ± 0.9</td>
<td>98.9 ± 4.2</td>
<td>104.4 ± 6.5</td>
<td>*80.6 ± 2.7</td>
<td>108.4 ± 3.1</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; (10&lt;sup&gt;-3&lt;/sup&gt;M)</td>
<td>*77.5 ± 3.0</td>
<td>94.2 ± 3.4</td>
<td>97.5 ± 3.4</td>
<td>*89.3 ± 1.9</td>
<td>108.9 ± 3.4</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; (10&lt;sup&gt;-4&lt;/sup&gt;M)</td>
<td>*63.9 ± 4.4</td>
<td>99.7 ± 5.0</td>
<td>104.1 ± 3.0</td>
<td>*82.6 ± 1.4</td>
<td>100.9 ± 3.5</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; (10&lt;sup&gt;-4&lt;/sup&gt;M)</td>
<td>*83.9 ± 3.9</td>
<td>93.6 ± 3.9</td>
<td>91.4 ± 5.4</td>
<td>93.5 ± 3.2</td>
<td>103.0 ± 3.2</td>
</tr>
</tbody>
</table>

*p < 0.05 compared to controls.

animals was found to be hypothyroid as judged by inhibition of weight gain and undetectable serum levels of T3. The uptake of <sup>1</sup>HIGABA in homogenates derived from these animals was significantly enhanced.

**DISCUSSION**

We have described for the first time a specific inhibition by T<sub>3</sub> and T<sub>4</sub> of the high affinity neuronal uptake of <sup>1</sup>HIGABA 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<sub>3</sub> and T<sub>4</sub> are in the low nanomolar range (Dratman et al., 1983), and therefore nearly three orders of magnitude below the IC<sub>50</sub> determined for T<sub>3</sub> and T<sub>4</sub>, 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 <sup>1</sup>HIGABA by T<sub>3</sub> or T<sub>4</sub> is physiologically concordant with the observed increase in <sup>1</sup>HIGABA uptake in cortical homogenates of hypothyroid rats; however, there is nothing
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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 $5 \times 10^{-6}$ to $5 \times 10^{-5}$M) in the presence of 0, 10$^{-4}$M, or $5 \times 10^{-4}$M T$_3$ (A), or 0, $5 \times 10^{-4}$M, or $3 \times 10^{-4}$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.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Body weights (g)</th>
<th>Serum T$_3$ levels (ng/dl)</th>
<th>Uptake of $[^3]H$GABA (% control ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation (Control)</td>
<td>6</td>
<td>241 ± 3.2</td>
<td>53 ± 4</td>
<td>100.0 ± 3</td>
</tr>
<tr>
<td>Thyroidectomy</td>
<td>6</td>
<td>162 ± 6.2*</td>
<td>&lt; 5 *</td>
<td>119.0 ± 5*</td>
</tr>
<tr>
<td>Sham operation + 500 µg/kg T$_3$</td>
<td>6</td>
<td>190 ± 7.3*</td>
<td>225 ± 22*</td>
<td>94.0 ± 3</td>
</tr>
<tr>
<td>Thyroidectomy + 500 µg/kg T$_3$</td>
<td>6</td>
<td>141 ± 6.4*</td>
<td>740 ± 39*</td>
<td>99.0 ± 5</td>
</tr>
</tbody>
</table>

*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, T1 and T2 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 (T1 > T2 > rT3) is the same as their order of potency in inhibiting the uptake of GABA. Our findings that T1 and T2 inhibit GABA uptake might represent a mechanism whereby thyroid-induced excitation would be limited: increased levels of T1 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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