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SHORT COMMUNICATIONS

Thyrotropin-releasing hormone—increased catabolism of catecholamines in brains of thyroidectomized rats

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Thyrotropin-releasing hormone (TRH) is a tripeptide (L-pyroglutamyl-L-histidyl-L-proline-amide), found in the hypothalamus, that functions as a hypophysiotropic hormone. Synthetic TRH exerts behavioral effects in man and animals, and has been administered to depressed patients [1–6]. Schildkraut [7] has proposed that antidepressive drugs produce their effect by increasing the functional activity of central catecholaminergic neurons of the brain; however, TRH does not mimic the effects of other known antidepressives that either block the re-uptake of monoamines [8] or inhibit monoamine oxidase [9]. The present study was undertaken to determine if administration of TRH increases the depletion of catecholamines after inhibition of synthesis by the administration of alpha-methyltyrosine (α-MT). This approach has previously been used to estimate the rate of catecholamine turnover in brain [10], and can be used to show that treatment with drugs affects the turnover rate of catecholamines. Because TRH may produce its effects through activation of the thyroid gland, the experiments were performed in both thyroidectomized and sham-operated control rats. While this study was underway, at least three other laboratories reported the effects of TRH on catecholamine metabolism in brain [11–13].

Male Sprague–Dawley rats (250–300 g), obtained from Charles River Laboratories, were used in all experiments. Thyrotropin-releasing factor was synthesized and generously provided by Dr. Miguel Ondetti, Peptides Section, The Squibb Institute for Medical Research. Alpha-methyltyrosine methyl ester (Aldrich Chemical Co.) and TRH were dissolved in 0.9% saline and administered intraperitoneally. Rats were decapitated, and the excised brains were dissected in ice water, as previously described [14]. Catecholamines were measured in homogenates of brain prepared in 0.4% perchloric acid. The catecholamines were isolated from the homogenate by adsorption onto alumina [10], and were oxidized and measured fluorometrically by the method of Chang [15], after their elution from the alumina into 0.1 N acetic acid. Endogenous concentrations of catecholamines were calculated from standards (1 nmole each) of dopamine and norepinephrine (Sigma Chemical Corp.) carried through the assay with the tissue samples.

In a preliminary experiment, we had found that the administration of TRH (15 or 30 mg/kg, i.p.) had no effect on the endogenous concentration of either dopamine or norepinephrine in the brains of rats sacrificed 0.5, 2, 4 or 5 hr after treatment. To determine the effect of TRH on the turnover of catecholamines, the peptide was administered to rats that had been treated with α-MT to inhibit synthesis of norepinephrine and dopamine. As shown in Table 1, treatment with α-MT alone caused depletion of both norepinephrine and dopamine in all brain regions studied, and the extent of this depletion was nearly the same in both sham-operated and thyroidectomized rats. However, in rats subsequently treated with TRH, α-MT caused a significantly greater depletion of norepinephrine in both the cortex and in the "Rest of brain" (whole brain less cortex, cerebellum and corpus striatum), and of dopamine in the cortex, corpus striatum and "Rest of brain" than it had in rats given only α-MT. Both sham-operated control and thyroidectomized animals showed this increase in the rate of depletion of dopamine and norepinephrine induced by the administration of TRH.

The results of this study demonstrate that administration of TRH potentiates the depletion in brain of norepinephrine and dopamine resulting from the inhibition of their synthesis by the administration of α-MT. The rate of depletion of catecholamines after inhibition of synthesis provides an index of the rate of turnover of catecholamines, if TRH treatment does not affect the steady-state concentration of catecholamines in the time interval during which depletion is measured. Because the administration of TRH had no effect on levels of endogenous catecholamines, our results indicate that TRH increases the rate of turnover of dopamine and norepinephrine. The increase in dopamine and norepinephrine turnover occurred in brains of both sham-operated control rats and of thyroidectomized rats. The latter finding suggests that the increase in catecholamine turnover induced by the administration of TRH is independent of any effect of TRH on the thyroid gland.

While the present study was underway, three other laboratories reported the effects of the administration of TRH on the metabolism of catecholamines in rat brain. All of these investigators found that administration of TRH had no effect on the concentration of catecholamines. Keller *et al.* [11] did find that the administration of TRH (10 mg/kg, i.p.) to rats induced an increase in concentration of the norepinephrine metabolite, 3-methoxy-4-hydroxyphenylethyleneglycol (MOPEG), and accelerated the rate of conversion of radiolabeled tyrosine to norepinephrine. The increase in concentration of MOPEG also occurred in thyroidectomized rats. Reigle *et al.* [12] found that the administration of TRH (8 mg/kg, i.p.) caused a slight increase in the rate of efflux of radiolabeled norepinephrine from the brain after intracisternal administration of the isotope. Using alpha-methyltyrosine to measure the rate of turnover of brain catecholamines, Horst and Spirt [13] also found an increase in the norepinephrine turnover rate after treatment with TRH. Thus, we have confirmed the previous findings of a TRH-induced increase in norepinephrine metabolism in the brain. However, in contrast to our results, neither Keller *et al.* [11] nor Horst and Spirt [13] found an increase in the turnover rate of dopamine in brains of rats treated with TRH. This discrepancy may be explained by the fact that a smaller dose of TRH (10 mg/kg) was used in other studies.

The increase in the turnover rate of catecholamines may be related to the antidepressive effect of TRH reported by several investigators. The tricyclic antidepressive agents when administered to rats in a single dose cause a reduction in the rate of norepinephrine turnover; however, when

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Table 1. Effect of thyrotropin-releasing factor on the depletion of brain catecholamines induced by α-methyltyrosine*

Group No.	Treatment: Thyroid-ectomy	α-MT	TRH	Dopamine concn (nmoles/g ± S. E.): Cortex	Corpus striatum	Rest of brain	Norepinephrine concn (nmoles/g ± S. E.): Cortex	Rest of brain
1	0	0	0	2.72 ± 0.06	28.4 ± 0.9	4.11 ± 0.18	1.37 ± 0.06	2.96 ± 0.07
2	0	+	0	2.01 ± 0.06	18.2 ± 0.6	2.61 ± 0.09	0.94 ± 0.04	1.54 ± 0.05
3	+	0	0	2.74 ± 0.06	29.4 ± 0.9	4.25 ± 0.25	1.40 ± 0.08	3.00 ± 0.10
4	+	+	0	2.15 ± 0.06	20.5 ± 0.6	2.48 ± 0.11	0.89 ± 0.03	1.46 ± 0.04
5	0	0	+	2.88 ± 0.09	29.1 ± 0.6	3.99 ± 0.22	1.41 ± 0.06	2.98 ± 0.09
6	0	+	+	1.47 ± 0.05†	12.7 ± 0.4‡	1.84 ± 0.08§	0.58 ± 0.03‖	1.30 ± 0.04¶
7	+	0	+	2.77 ± 0.08	28.0 ± 1.1	3.94 ± 0.17	1.34 ± 0.05	2.87 ± 0.09
8	+	+	+	1.60 ± 0.05**	14.7 ± 0.5††	1.69 ± 0.09‡‡	0.62 ± 0.01§§	1.19 ± 0.04‖‖

* Thyrotropin-releasing hormone (TRH; 15 mg/kg, i.p.) was administered to rats 1.5 and 2.5 hr after treatment with α-methyltyrosine (α-MT; 400 mg/kg, i.p.). Rats were killed 3 hr after α-MT administration. Values are the means for five or six animals. "Rest of brain" refers to the whole brain, excluding cortex, cerebellum and corpus striatum. There were no differences ($P > 0.05$) between sham-operated and thyroidectomized control rats (group 1 vs 3), sham-operated and thyroidectomized rats treated with α-MT (group 2 vs 4) or with TRH alone (group 5 vs 7), or between sham-operated and thyroidectomized rats treated with both α-MT and TRH (group 6 vs 8).

† $P < 0.01$ compared with dopamine content in cortex of rats treated with α-MT alone (group 2).

‡ $P < 0.01$ compared with dopamine content in corpus striatum of rats treated with α-MT alone (group 2).

§ $P < 0.01$ compared with dopamine content in "Rest of brain" of rats treated with α-MT alone (group 2).

‖ $P < 0.01$ compared with norepinephrine content in cortex of rats treated with α-MT alone (group 2).

¶ $P < 0.01$ compared with norepinephrine content in "Rest of brain" of rats treated with α-MT alone (group 2).

** $P < 0.01$ compared with dopamine content in cortex of thyroidectomized rats treated with α-MT alone (group 4).

†† $P < 0.01$ compared with dopamine content in corpus striatum of thyroidectomized rats treated with α-MT alone (group 4).

‡‡ $P < 0.05$ compared with dopamine content in "Rest of brain" of thyroidectomized rats treated with α-MT alone (group 4).

§§ $P < 0.01$ compared with norepinephrine content in cortex of thyroidectomized rats treated with α-MT alone (group 4).

‖‖ $P < 0.01$ compared with norepinephrine content in "Rest of brain" of thyroidectomized rats treated with α-MT alone (group 4).

these compounds are given chronically to rats, they stimulate the release and rate of turnover of norepinephrine [16]. The delay in the onset of the increase in catecholamine turnover induced by the tricyclic compounds correlates with the delay in onset of antidepressive activity in patients. The fact that TRH augments the rate of turnover of catecholamines after acute treatment may explain its immediate therapeutic effect in some depressed patients, in contrast to the delayed onset of activity of the tricyclic compounds.

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