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BRIEF COMMUNICATION

Lack of Effect of Chronically Administered Thyrotropin-Releasing Hormone (TRH) on Regional Rat Brain Tyrosine Hydroxylase Activity

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NEMEROFF, C. B., J. A. DIEZ, G. BISSETTE, A. J. PRANGE, JR., L. E. HARRELL, AND M. A. LIPTON. *Lack of effect of chronically administered thyrotropin-releasing hormone (TRH) on regional rat brain tyrosine hydroxylase activity.* PHARMAC. BIOCHEM. BEHAV. 6(4) 467–469, 1977. – Chronic treatment of adult male rats with TRH (1 or 10 mg/kg IP) for 5 or 9 days failed to alter the activity of tyrosine hydroxylase (TH), the enzyme regulating the rate-limiting step in catecholamine biosynthesis. In contrast, as previously described, chronic reserpine administration (0.5 mg/kg IP; 9 days) resulted in a significant rise in TH activity in midbrain, hypothalamus, pons-medulla and forebrain. These results suggest that the enhanced brain norepinephrine turnover reported to occur after treatment with TRH is not due to synthesis of new TH enzyme protein.

TRH Reserpine Tyrosine hydroxylase

SEVERAL studies have indicated that the tripeptide, pGlu-His-Pro-NH_2 (thyrotropin-releasing hormone; TRH) exerts direct central nervous system effects apparently independent of its action on the adenohypophysis [15, 25, 26]. For example, TRH is a potent antagonist of barbiturate- and ethanol-induced sedation and hypothermia [2, 4, 24]. In addition TRH potentiates the stimulant properties of L-DOPA in pargyline-pretreated mice [12,20], a test devised by Everett [9] to screen antidepressant drugs. These effects are clearly not mediated via the pituitary-thyroid axis since neither hypophysectomy nor thyroidectomy abolish these neurotrophic actions of the tripeptide; and, furthermore, treatment with thyroid hormones or thyrotropin are ineffective in these paradigms [26]. TRH has also been reported to possess antidepressant activity in man [1, 13, 17, 22, 23], though controversy currently surrounds this issue [6,18].

Several investigators have studied the effects of TRH on brain catecholamine systems. Although TRH administration does not alter endogenous brain levels of norepinephrine, dopamine or serotonin [3, 11, 21, 27], both biochemical [14] and histochemical [5] estimates reveal that the tripeptide, administered peripherally, results in increased brain norepinephrine turnover. The purpose of the present study was to evaluate the effect of chronic treatment with TRH on regional rat brain tyrosine hydroxylase (TH) activity. This enzyme controls the rate-limiting step in catecholamine biosynthesis [30] and changes in its activity might explain the enhanced brain norepinephrine turnover that has been reported after TRH administration.

METHOD

Adult male Sprague-Dawley rats (350 g) were purchased from Holtzman Laboratories (Madison, WI) and maintained in a controlled lighting air-conditioned animal facility. They received laboratory chow and water ad lib. The rats were treated daily with TRH (1 or 10 mg/kg IP) for 5 or 9 days, reserpine (0.5 mg/kg IP), or reserpine (0.5 mg/kg, IP) + TRH (10 mg/kg IP) for 9 days, or vehicle (0.9% NaCl, pH 7.5). The inclusion of a reserpine group was based on the fact that animals so treated show an induction of TH activity [28] and could, therefore, serve as a positive control group. Animals were sacrificed 24 hr after their last drug treatment.

TABLE 1

THE EFFECT OF CHRONIC TREATMENT WITH THYROTROPIN-RELEASING HORMONE (TRH) FOR 5 DAYS ON REGIONAL RAT BRAIN TYROSINE HYDROXYLASE ACTIVITY*

Treatment	Hypothalamus	Midbrain	Pons-medulla	Forebrain
Saline	100 ± 4.1	100 ± 1.3	100 ± 1.7	100 ± 0.8
TRH 1mg/kg	104 ± 8.3	102 ± 4.8	107 ± 4.6	107 ± 3.3
TRH 10mg/kg	101 ± 7.3	110 ± 3.9	102 ± 9.1	100 ± 5.7

*Tyrosine hydroxylase activity is expressed as a percentage of mean activity in at least 6 saline-injected controls. Each experimental point represents the mean ± SEM of 5 animals.

TH activity was assessed by a modification of the method of Nagatsu *et al.* [19] utilizing 6-MPH_4 as the cofactor. Protein was determined by the method of Lowry *et al.* [16]. Enzyme activity was estimated in 4 brain regions (hypothalamus, midbrain, forebrain and pons-medulla) utilizing a modification of the dissection technique of Glowinski and Iversen [10].

Inhomogeneity of variance and unequal cell size between the various experimental and control groups led to a nonparametric statistical evaluation of the data; the Mann-Whitney U test was utilized [29]. Data are expressed either as TH activity (nmoles/hr/mg protein) or as a percentage of control enzyme activity.

RESULTS AND DISCUSSION

Chronic treatment with TRH (1 or 10 mg/kg) for either 5 or 9 days did not alter TH activity in any of the brain regions examined (Tables 1 and 2). As previously reported [28] reserpine treatment for 9 days resulted in a significant increase in TH activity in all of the brain regions assayed. The results also demonstrate that chronic TRH treatment does not alter the reserpine-induced enhancement in brain TH activity. In addition TRH treatment did not change the characteristic loss of body weight observed in the reserpinized animals (data not shown). In preliminary studies, no change in TH activity was observed after acute TRH administration.

These results suggest that changes in the activity of brain TH as measured in the *in vitro* enzyme assay system utilized, are not responsible for the increased norepinephrine turnover reported to occur in TRH-treated animals [5,14]. The paradoxical finding that TRH does not alter brain TH activity while increasing brain norepinephrine turnover may be explained by allosteric changes in the enzyme protein induced by the tripeptide which are undetectable with the assay systems employed. Other workers, however, recently have suggested that TRH interacts with brain cholinergic and gabergic systems [7,8].

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TABLE 2

THE EFFECT OF CHRONIC TREATMENT WITH THYROTROPIN-RELEASING HORMONE (TRH) OR RESERPINE FOR NINE DAYS ON REGIONAL RAT BRAIN TYROSINE HYDROXYLASE ACTIVITY

Treatment	Tyrosine Hydroxylase Activity (nmoles/hr/mg protein ± SEM) Hypothalamus	Midbrain	Pons-medulla	Forebrain
Saline	1.15 ± 0.04 (n=26)	1.74 ± 0.07 (n=25)	0.423 ± 0.015 (n=23)	2.80 ± 0.08 (n=26)
TRH 1mg/kg	1.19 ± 0.08 (n=5)	1.66 ± 0.21 (n=5)	0.444 ± 0.033 (n=5)	2.42 ± 0.30 (n=5)
TRH 10mg/kg	1.21 ± 0.04 (n=19)	1.65 ± 0.095 (n=19)	0.404 ± 0.019 (n=18)	2.76 ± 0.12 (n=19)
Reserpine 0.5mg/kg	1.35 ± 0.05* (n=15)	1.95 ± 0.08* (n=15)	0.602 ± 0.026‡ (n=15)	3.24 ± 0.12† (n=15)
TRH 10mg/kg *plus* Reserpine 0.5mg/kg	1.27 ± 0.07 (n=7)	1.90 ± 0.06* (n=7)	0.604 ± 0019‡ (n=7)	3.11 ±0.15* (n=7)

*$p<0.05$.
†$p<0.0025$ Mann Whitney U Test.
‡$p<00001$.

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