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Thyrotropin Releasing Hormone (TRH): DOPA Potentiation and Biogenic Amine Studies

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PLOTNIKOFF, N. P., G. R. BREESE AND A. J. PRANGE, JR. *Thyrotropin releasing hormone (TRH): DOPA potentiation and biogenic amine studies.* PHARMAC. BIOCHEM. BEHAV. 3(4) 665–670, 1975. – The present study in mice demonstrated that TRH when administered over 5 days remained active in the Everett Dopamine Potentiation Test. No evidence of tolerance was observed. In fact, an accumulative effect of TRH appeared to take place. Ablation of the adrenals, ovaries, testes, pineal, spleen, parathyroid, one kidney, or thymus did not disrupt this behavioral potentiation of dopamine by TRH. TRH was found to potentiate the effects of imipramine. T3, T4, and TSH were found to be active in the DOPA potentiation test. No overt toxicity was observed between TRH and pargyline or between TRH and DOPA. Toxicity was seen only when all three agents were used together. TRH was found active in young and old mice. TRH was also found active in potentiating the central effects of serotonin. Biogenic amine brain levels in mice were not altered by TRH when administered for five days. Alpha-methyl-p-tyrosine reduced the activity of TRH in the dopamine potentiation test, suggesting dopaminergic mechanisms are involved by a direct receptor interaction.

TRH DOPA Biogenic amines

THE finding by our group that thyrotropin releasing hormone (TRH) is active in the pargyline-DOPA mouse activation test formed in part the motive for trials of the hormone as a remedy for depression [16]. Prange and Wilson [19,20] showed that TRH has a fast onset of antidepressant activity. This finding was confirmed by Kastin, *et al.* [10] and Vander *et al.* [23]. However, more recent reports failed to confirm the earlier studies [4, 13, 22].

With these clinical findings at hand, it became a matter of interest to examine the activity of TRH in more detail on the behavioral response of DOPA and to examine the activity of related substances in this test. In the course of this work, it was rational to include in addition to the DOPA test, an animal test that relates to cerebral indoleamines because these substances have also been implicated in the pathogenesis of depression [3, 6, 12]. The final phase of the present work was to examine the influence of TRH on levels of cerebral biogenic amines in the brain.

METHOD

Behavioral Studies

DOPA potentiation test. The Everett DOPA/pargyline Potentiation Test was used in the present study [5]. The method consists of pretreating mice (17–22 g) with pargyline HCl orally (40 mg/kg), at least 2 hr before administering TRH. After pargyline and TRH treatment, dl DOPA (200 mg/kg) is given intraperitoneally and the animals (4 per cage) observed for 1 hr. One to 3 cages were employed for each dose. Potentiation of the DOPA-induced response was scored as marked increase in irritability and reactivity, jumping, squeaking, and aggressive fighting. The Swiss albino mice used in these behavioral studies and ablated animals were obtained from the Charles River Laboratories and the Altech Laboratories, Madison, Wisconsin.

Serotonin potentiation test. Just as DOPA is the amino acid precursor of the catecholamines, so is 5-hydroxy-

tryptophan (5-HTP) the proximate precursor for serotonin. Because of the findings with DOPA, the effect of TRH on the biological responses to pargyline (40 mg/kg, PO) plus 5-HTP (100 mg/kg, IP) were studied. Characteristically, a syndrome develops which includes tremors, head movements, abduction of the limbs, and irritability. The effects are arbitrarily graded at 1 (for slight behavioral effects, 2 (for moderate effects), and 3 (for marked effects).

Neurochemical studies. Three to 4 groups (4 mice per dose) were used for each dose in the determination of brain biogenic amines. ICR strain male mice (17–22 g) were used. The levels of dopamine, norepinephrine, and serotonin were determined by techniques previously reported from this laboratory [5].

RESULTS

Behavioral Studies

No loss of activity or formation of tolerance to the behavioral potentiation of DOPA by TRH (Table 1) was observed. Thus, in a wide dose range (0.1 to 0.6 mg/kg) TRH was found to be active in the Everett DOPA Potentiation Test when administered daily for five days. Indeed, there appeared to be an accumulative effect resulting in greater potency on the fifth day.

Ablation Studies

Table 2 shows that intraperitoneal administration of TRH is effective in female mice. It also shows that activity of TRH is not lost in ovariectomized mice. By comparison with Table 3 it can be seen that there is a tendency for TRH to be more potent in female (0.1 mg/kg) than in male mice (0.4 mg/kg) in the pargyline-DOPA test. By these criteria, it is more potent in normal female than ovariectomized mice as well. In a series of ablation studies, TRH was found to be active in the DOPA test in adrenalectomized, thymectomized, unilaterally nephrectomized, splenectomized, parathyroidectomized, castrated, and pinealectomized mice (Table 3). TRH appeared to be more active in parathyroidectomized, castrated, and pinealectomized mice than in normal intact male mice.

TRH plus imipramine. In the present body of work, the pargyline-DOPA test was used for a final time to discern whether TRH would amplify the well known effects of imipramine in this test model. Table 4 confirmed once again the activity of TRH and the activity of imipramine. Furthermore, it shows that the activity of the two substances potentiate or at least add to each other's effects (Table 4).

Serotonin potentiation by TRH. Table 5 shows that TRH is active in the serotonin potentiation test. However, when TRH and imipramine were given togehter, no potentiation was observed. Imipramine at the highest dose tested 80 mg/kg, showed only moderate activity.

T3, T4, and TSH in the DOPA potentiation test. Table 6 clearly shows that T3, T4, and TSH are active in the DOPA potentiation test.

Toxicity studies in mice. In Table 7 are shown data of interaction effects between TRH, pargyline, and dl DOPA. When all substances are used together in the DOPA potentiation test, approximately half the mice die overnight in coma (Group 1), however, no toxicity was observed between pargyline and TRH (Group 2). Also, no toxicity was seen between TRH and DOPA (Group 3) in overnight studies.

TRH in older mice. TRH was found active in older mice (24 to 30 g) in the DOPA potentiation test. However, it was necessary to increase threshold by increasing the dose of pargyline from 40 to 45 mg/kg to demonstrate activity (Table 8).

Alpha-methyl-p-tyrosine and TRH. Pretreatment of mice with alpha-methyl-p-tyrosine markedly reduced the activity of TRH in the DOPA potentiation test but did not abolish it (Table 9).

TABLE 1

EFFECTS OF TRH ON DOPA RESPONSE IN PARGYLINE-TREATED MICE AFTER SINGLE AND MULTIPLE DOSE ADMINISTRATION

Drug	Dose mg/kg (oral route)	Single dose	Chronic (5 days)
Control	1	1	1
TRH	0.1	1	3
	0.2	1	3
	0.4	2	3
	0.8	3	3
	1.6	–	3

TABLE 2

EFFECT OF INTRAPERITONEAL TRH ON INTACT AND OVARIECTOMIZED FEMALE MICE IN THE DOPA POTENTIATION TEST*

TRH (mg/kg)	Behavioral rating			
Intact female mice				
0.05		2	1	
0.1		3	3	
0.2		3	2	
0.4		3	3	
0.8		3	3	
1.6		3	3	
Ovariectomized mice				
0.05	1	1	1	
0.1	1	1	1	
0.2	1	2	1	2
0.4	2	2	2	2
0.8	3	3	2	3
1.6	3	3	3	3

*Animals were treated and rated as in Table 1. TRH was administered 1 hr before DOPA. Rows of numbers indicate different experiments.

TABLE 3

DOPA POTENTIATION BY TRH IN ABLATED MALE MICE

Surgical procedure	Dose mg/kg IP route	Behavioral rating at 1 hr
None (intact)	0.05	1
	0.1	1
	0.2	2
	0.4	3
	0.8	3
Adrenalectomy	0.05	1
	0.1	2
	0.2	2
	0.4	3
	0.8	3
Thymectomy	0.05	1
	0.1	2
	0.2	2
	0.4	3
	0.8	3
Nephrectomy (unilateral)	0.05	1
	0.1	1
	0.2	2
	0.4	3
	0.8	3
Splenectomy	0.05	1
	0.1	1
	0.2	2
	0.4	3
	0.8	3
Parathyroidectomy	0.05	2
	0.1	3
	0.2	3
	0.4	3
	0.8	3
Castration	0.05	1
	0.1	2
	0.2	3
	0.4	3
	0.8	3
Pinealectomy	0.1	2
	0.2	3
	0.4	3
	0.8	2

The effects of TRH on brain biogenic amines. The above work strongly suggested an interaction between TRH and brain biogenic amines. For this reason it was of interest to examine the effects of TRH treatment on the levels of these substances. Groups of male Swiss-Webster mice were treated with various doses of IP TRH for 5 days or with controlled injection. Table 10 shows that none of the doses employed had a reliable effect on brain dopamine, norepinephrine, or serotonin. It can be seen that the behaviorally active doses of TRH were without effect on brain amines.

TABLE 4

POTENTIATION OF BEHAVIORAL EFFECTS OF DOPA IN MICE BY TRH AND IMIPRAMINE

Imipramine dose mg/kg (oral)	Behavioral rating: Imipramine alone	Behavioral rating: Imipramine plus TRH (0.1 mg/kg IP)
0.5	1	1
1	1	2
2	1	2
2.5	1	3
5	1	3
10	2	3
20	2	3

TABLE 5

EFFECTS OF TRH ON THE MOUSE 5-HTP POTENTIATION TEST

IP Route TRH alone		Oral Route Imipramine alone		Imipramine plus TRH*	
mg/kg	Behavioral rating	mg/kg	Behavior	mg/kg	Behavior
0.1	1	10	1	10	1
0.2	2	20	1	20	1
0.4	2	40	1	40	1
0.8	3	80	2	80	3

The doses of TRH were adminstered 1 hr prior to DL-5-HTP (100 mg/kg IP). All mic received pargyline (40 mg/kg, p.o.) 2 hr before TRH. The behavioral rating was based on a syndrome of tremors, head movements, abduction of limbs, and irritability and graded 1+ slight, 2+ moderate, and 3+ marked.

*Dose of TRH was 0.1 mg/kg which produced a 1+ slight effect.

DISCUSSION

It can be readily seen that the four hormones of the hypothalamic-pituitary-thyroid axis are active in the pargyline-DOPA test in mice. So far as has been discerned, TRH is active in both sexes. In female mice, the actions of TRH were not dependent upon the presence of the ovary. Furthermore, the activity of TRH depends neither upon the pituitary gland, nor on the thyroid gland. Nevertheless, T3, T4, and TSH were active in the test. Thus, the possibility remains that increased secretion of these substances after TRH could sustain the antidepressant effect of the hypothalamic hormone. TRH was also active in a test that relates

TABLE 6

EFFECT OF T3, T4, AND TSH ON THE RESPONSE TO DOPA IN PARGYLINE TREATED MICE

IP route (mg/kg)	Degree of behavioral potentiation	
	1 hr	4 hr
T3		
2.0	2	2
4.0	3	2
8.0	3	3
T4		
2.0	1	1
4.0	1	2
8.0	3	3
TSH		
2.0	1	3
4.0	2	3
8.0	2	3

TABLE 7

CONTROL TOXICITY STUDIES IN MOUSE DOPA POTENTIATION TEST

Group		Treatment	24 hr Mortality
1. Pargyline (oral)	Plus	TRH (IP) plus DOPA (IP)	
40 mg/kg		0.05 mg/kg	4/8
40 mg/kg		0.1 mg/kg	4/8
40 mg/kg		0.2 mg/kg	4/8
40 mg/kg		0.4 mg/kg	4/8
40 mg/kg		0.8 mg/kg	4/8
Control			0/8
2. Pargyline (oral)		TRH (IP)	
40 mg/kg		1 mg/kg	0/4
40 mg/kg		10 mg/kg	0/4
40 mg/kg		100 mg/kg	0/4
3. TRH (IP)		DOPA (IP)	
100 mg/kg		200 mg/kg	0/4
100 mg/kg		400 mg/kg	0/4
100 mg/kg		800 mg/kg	0/4

TABLE 8

TRH IN THE DOPA POTENTIATION TEST IN OLDER MICE*†

Group		Treatment	Behavioral rating at 1 hr
Pargyline 40 mg/kg	Plus	TRH (IP) mg/kg	
		0.2	1
		0.4	1
		0.8	2
		1.6	2
		Control	1
Pargyline 45 mg/kg	Plus	TRH (IP)	
		0.2	1
		0.4	2
		0.8	3
		1.6	3
		Control	1

*Mice 24–30 g body weight.
†TRH plus dl DOPA (200 mg/kg IP).

TABLE 9

EFFECT OF ALPHA-METHYL-P-TYROSINE ON TRH IN THE DOPA POTENTIATION TEST

TRH* IP mg/kg	Behavioral rating at 1 hr
0.4	1
0.8	2
1.6	3

*Pretreatment with alpha-methyl-p-tyrosine 300 mg/kg IP 4 hr prior to TRH administration.

TABLE 10

EFFECT OF TRH ON THE BRAIN BIOGENIC AMINES IN MICE*

	μg/g		
Oral route	Dopamine	Norepinephrine	Serotonin
Controls	0.91 ± 0.02	0.59 ± 0.01	0.55 ± 0.02
TRH			
0.5 mg/kg	0.96 ± 0.02	0.61 ± 0.01	0.56 ± 0.05
1.0 mg/kg	0.94 ± 0.03	0.57 ± 0.02	0.52 ± 0.01
2.0 mg/kg	0.91 ± 0.01	0.55 ± 0.01	0.55 ± 0.02
4.0 mg/kg	1.01 ± 0.02	0.55 ± 0.01	0.51 ± 0.02
8.0 mg/kg	0.93 ± 0.01	0.55 ± 0.01	0.54 ± 0.01

*Animals were treated with various doses of TRH for 5 days and sacrificed 1 hr after the last dose of TRH.

to the behavioral effects of indoleamines. Furthermore, when TRH was given daily for 5 days, there appeared an accumulative effect as judged by the results in the dopamine potentiation test. The present studies indicate that removal of the adrenals. thymus, one kidney, spleen, parathyroid, testes, ovaries, or pineal gland did not prevent the activity of TRH in the dopamine potentiation test. However, the potency of TRH was reduced in ovariectomized and increased in castrated, parathyroidectomized, or pinealectomized mice compared to normal intact mice. Thus, there appear to be interactions between TRH and the secretions of these glands which will require further study.

The antidepressant activities of TRH as well as imipramine appear to enhance each other in the dopamine potentiation test. However, the mechanisms of the two substances may be quite different. Recently, Breese, *et al* [1] found no effect of TRH on uptake of H^3-norepinephrine in rat brain. This finding is in sharp contrast to imipramine which has significant effects on synaptic uptake and metabolism of amines. Furthermore, present findings are consistent with those of Breese, *et al,* [1] that TRH does not elevate brain levels of dopamine, norepinephrine, and serotonin. Dopaminergic receptors are probably affected since our studies showed alpha-methyl-p-tyrosine reduced the effects of TRH in the DOPA test and dopamine has been shown to be elevated in TRH treated mice given DOPA after pargyline [1].

In addition, Keller *et al.* [1], Breese, *et al.* ([2], as well as Horst and Spirt [7]), have reported that TRH increases the rate of norepinephrine turnover and release in brain tissue. This may be consistent with the finding of Reigle *et al.* [14] who reported that TRH produced an increase in brain levels of tritiated normetanephrine in rats.

Recently, Plotnikoff *et al.* [14,15] reported that MIF also potentiates the actions of DOPA and at the same time does not elevate brain levels of dopamine, norepinephrine, and serotonin. These findings with TRH and MIF in the DOPA test were confirmed by Huidobro-Toro *et al.* [8]. However, by way of contrast, MIF has been reported not to be active in the 5-HTP test (Plotnikoff). In another place we have suggested that both serotonin and catecholamines are involved in the chemical pathogenesis of depression, and that in depression both groups of substances are deficient [17,18].

It is also of interest to note that MIF has been found useful in the treatment of Parkinson's disease [9] while TRH appears to be inactive. These findings are consistent with the view that in Parkinson's disease a disruption of the normal balance of amines is involved [18]. Thus, peptides which promote catecholamine activity and/or indoleamine activity might reasonably be expected to have activity in psychiatric and/or neurological disorders. In this regard, no toxic interactions were observed between pargyline and TRH or between DOPA and TRH. Toxicity was observed only when all three substances were administered to test animals. In addition we found that the level of threshold changes of monoamino-oxidase inhibition was critical in relationship to age. The older mice required a higher dose of pargyline to produce behavioral potentiation of dopamine by TRH. It appears that critical levels of biogenic amines are required for behavioral expression of TRH effects. It is possible that TRH may be increasing the sensitivity of dopaminergic receptor systems.

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