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Antagonism of Isolation-Induced Aggression in Mice by Thyrotropin-Releasing Hormone (TRH)

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MALICK, J. B. *Antagonism of isolation-induced aggression in mice by thyrotropin-releasing hormone (TRH).* PHARMAC. BIOCHEM. BEHAV. 5(6) 665–669, 1976. – TRH was shown to be an extremely potent (ED50 = 0.04 mg/kg, IP) antagonist of isolation-induced aggression in male mice. The antifighting activity of TRH was selective in that it did not produce concurrent neurological impairment or significant alterations in spontaneous locomotor activity at antiaggressive doses. This activity of TRH appeared to be a direct affect on CNS structures since neither triiodothyronine nor any of the constituent amino acids of TRH antagonized aggression in isolated mice. The results are discussed in terms of the recent clinical effectiveness of TRH in some cases of mental illness (e.g., depression and schizophrenia).

Thyrotropin-releasing hormone (TRH) Isolation-induced aggression Triiodothyronine Antiaggressive activity Spontaneous motor activity d-Amphetamine SO_4

THYROTROPIN-releasing hormone (TRH; L-pyroglutamyl-L-histidyl-L-prolineamide) is a hypothalamic releasing factor which produces an increased output of thyroid-stimulating hormone (TSH) from the pituitary [25]. However, in recent years many reports have led to the conclusion that TRH, in addition to its effect on the pituitary-thyroid axis, also may have direct actions on the central nervous system (CNS).

TRH has been shown to potentiate the stimulant activity of dihydroxyphenylalanine (DOPA) in pargyline-pretreated mice [16]; this was the first demonstration that this tripeptide had effects on the CNS. This activity of TRH appeared to be independent of its activity on either the pituitary or thyroid glands since the potentiation still occurred in hypophysectomized mice [17] and in thyroidectomized rats [17].

Prange and Wilson and their co-workers [18,19] originally reported that TRH exhibited rapid, short-lasting antidepressant activity in man. Subsequently, this finding has been confirmed by several authors [12, 15, 26]; however, some conflicting negative findings with TRH in depression have been reported [2, 7, 22]. TRH has also been shown to produce mild euphoria and a sense of relaxation in normal women which appeared to be unrelated to thyroid stimulation [27,28] and other therapeutic effects in cerebral gigantism [23] and hyperkinesia in children [24]. In addition, significant efficacy in the treatment of schizophrenia has been reported [8,28]; however, Clark and co-workers [6] have failed to replicate this finding in long-term institutionalized chronic schizophrenic patients.

Thus, TRH appears to act on CNS structures to affect a wide range of mental states and behaviors. The purpose of the present study was to investigate the effects of TRH on the fighting behavior of mice induced by prolonged periods of social isolation.

EXPERIMENT 1: EFFECTS OF TRH ON ISOLATION-INDUCED AGGRESSION IN MICE

Method

Male CF No. 1-S mice (18–22 g) were made aggressive by a modification of the method of Yen and co-workers [30] which has been reported previously [1]. Briefly, mice were isolated for a period of 4 weeks and then tested for aggression by placing an isolated mouse into the home cage of another isolate. Pairs of mice were observed for 3 min, and presence or absence of fighting was recorded on an all-or-none basis. All pairs of mice were tested for aggression on the day before drug administration and only those which were consistent fighters were used for drug studies. Mice were tested for aggression at 30 min and 3 hr postdrug administration. Different groups of mice were used for each dose of drug, i.e., mice were only tested once following drug administration. Immediately following each aggression test, the mice were checked for neurological impairment by

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gently placing them on a 45° inclined screen; any mouse that exhibited impaired performance was scored as being ataxic.

All drugs were dissolved in distilled water and were administered via the intraperitoneal (IP) route in a volume of 10 ml/kg. The TRH used in this study was purchased from Chemical Dynamics Corp., South Plainfield, NJ. The ED50, that dose which would be expected to prevent fighting in 50% of the pairs, and the 95% fiducial limits were calculated by Probit Maximum Likelihood Analysis [10]; according to this method, percent responders were fitted against the log dose of drug in order to calculate the ED50.

Results and Discussion

Table 1 summarizes the results of this experiment. TRH was found to be an extremely potent (ED50 = 0.04 mg/kg, IP) antagonist of isolation-induced aggressive behavior at 30 min postdrug administration. The antiaggressive activity of TRH was significantly reduced but still prominent at 3 hr postdrug administration. TRH was found to be a selective antagonist of fighting at both time periods since none of the mice exhibited any signs of neurological or neuromuscular impairment on the inclined screen at any of the doses tested (highest dose tested was 100 mg/kg, IP).

EXPERIMENT 2: EFFECTS OF TRIIODOTHYRONINE AND THE CONSTITUENT AMINO ACIDS OF TRH ON ISOLATION AGGRESSION

Method

Since TRH stimulates the thyroid gland via the release of thyroid stimulating hormone (TSH), it was possible that the resultant increase in secretion of thyroid hormone (e.g., triiodothyronine; T_3) was responsible for the antiaggressive effects observed following TRH administration. To test this hypothesis, T_3 was administered to isolated mice at a relatively high dose (50 μg/kg), and the mice were tested for aggressive behavior as in Experiment 1.

In addition, another possible explanation for the effects observed following TRH administration was that the tripeptide was being catabolized and that one of the constituent amino acids was responsible for the behavioral inhibition. Thus, each of the three constituent amino acids of TRH (L-histidine, L-proline amide, and L-pyroglutamic acid) were tested, at doses higher than could have been achieved following catabolism of active doses of TRH, for their ability to antagonize isolation-induced aggression.

Results and Discussion

T_3 failed to antagonize the fighting of mice induced by prolonged social isolation (Table 2). Thus, it does not appear likely that the antiaggressive effects of TRH were mediated by secondary influences of TRH on the thyroid (e.g., release of thyroid hormones). In a previous study, treatment for 7 consecutive days with another thyroid hormone, thyroxine (2 mg/kg, IP), significantly reduced the length of time in isolation necessary to induce aggression whereas treatment with an antithyroid drug, thiouracil (25 mg/kg, IP) significantly extended the induction period [31]. In addition, if mice were hypophysectomized prior to isolation they failed to fight [31]. Thus, if anything, one would expect TRH to enhance aggression. Therefore, it appeared that TRH was antagonizing aggression via mechanisms that were not related to its influence on the pituitary-thyroid axis.

In addition, none of the constituent amino acids of TRH significantly inhibited isolation-induced aggression in mice (Table 2). Thus, it appeared that the effects observed following the administration of TRH were due to the activity of the parent compound.

EXPERIMENT 3: EFFECTS OF TRH AND AMPHETAMINE ON SPONTANEOUS MOTOR ACTIVITY

TRH has been reported to significantly increase spontaneous motor activity following both intraventricular infusion (23 nmoles over 15 min) in rats [21] and intraperitoneal administration (5 mg/kg) in mice [3]. These reports suggested that TRH may possess amphetamine-like activity.

The present studies were designed to determine whether possible amphetamine-like properties of TRH could have been responsible for the inhibition of aggression observed in isolated mice. d-Amphetamine sulfate was tested to determine whether it would selectively antagonize aggressive behavior in isolated mice. In addition, the effect of TRH on spontaneous motor activity was studied at doses which were observed to significantly antagonize fighting in order to determine whether overt motor stimulation may have interfered with fighting and produced a nonselective antagonism of aggression.

Method

d-Amphetamine sulfate was administered to isolated mice over a range of doses (1.0–5.0 mg/kg, IP) and aggressive behavior was studied as in Experiment 1 at 30 min postdrug.

The influence of TRH on spontaneous motor activity was studied at a dose approximately 2 times its ED50 for antagonism of aggression (0.1 mg/kg, IP) and at a dose approximately 20 times higher (1.0 mg/kg, IP). d-Amphetamine sulfate (1 and 3 mg/kg, IP) was used as a positive control. Spontaneous motor activity was measured utilizing a photocell chamber (Lehigh Valley Electronics). The chamber was circular, and the walls contained six equally spaced photocells. Each time a mouse broke a beam of light going to one of the photocells, one unit of motor activity was automatically recorded. Male mice, either isolated or group-housed controls, were injected with drug intraperitoneally and isolated one per cage for 30 min after which 4 mice were placed in the activity chamber and, total activity was measured for the next 15 minutes. This procedure was replicated 5 times for each drug dose, and the mean activity was calculated and compared to appropriate saline controls via a Student's *t*-test.

Results and Discussion

d-Amphetamine failed to antagonize fighting in any of the 10 pairs of isolated mice tested at each of three doses (1, 2.5, and 5 mg/kg, IP). Thus, antagonism of isolation-induced aggression by TRH was most likely not related to amphetamine-like activity.

The results of the motor activity study are summarized on Table 3. TRH failed to significantly alter spontaneous motor activity at any dose tested in either group-housed controls or isolated aggressive mice. Since TRH failed to significantly increase motor activity even at a dose at least

TABLE 1

ANTAGONISM OF ISOLATION-INDUCED AGGRESSION IN MICE BY TRH

Treatment (Time postdrug administration)	N*	ED50 (mg/kg, IP) for antagonism of isolation-induced fighting in mice (95% fiducial limits)†
TRH (30 min)	40	0.04 (0.01–0.7)
TRH (180 min)	40	2.8 (0.6–13.2)

*Number of pairs of mice tested.
†ED50 and 95% fiducial limits were calculated by Maximum Likelihood Probit Analysis (10).

TABLE 2

EFFECTS OF TRIIODOTHYRONINE (T_3) AND THE CONSTITUENT AMINO ACIDS OF TRH ON ISOLATION-INDUCED AGGRESSION IN MICE

Treatment	Dose (mg/kg, ip)	N*	%Antagonism of isolation induced aggression†
saline control	—	20	5
l-triiodothyronine	50 μg/kg	10	0
l-histidine	2.5	10	0
l-proline amide	2.5	10	0
l-pyroglutamic acid	2.5	10	10

*Number of pairs of mice tested.
†Mice tested 30 min postdrug administration.

TABLE 3

EFFECTS OF TRH AND AMPHETAMINE ON SPONTANEOUS MOTOR ACTIVITY IN GROUP-HOUSED OR ISOLATED MICE

		Motor Activity			
		Group-Housed		Isolated	
Treatment	Dose (mg/kg, ip)*	Mean ± SEM†	Statistical Significance‡	Mean ± SEM†	Statistical Significance‡
Saline	—	2041 ± 45	—	1663 ± 15	—
TRH	0.1	2081 ± 28	N.S.	1630 ± 61	N.S.
	1.0	2140 ± 71	N.S.	1712 ± 33	N.S.
d-Amphetamine SO_4	1.0	2359 ± 32	<0.01	1609 ± 52	N.S.
	3.0	2806 ± 57	<0.01	1771 ± 52	<0.05

*Five groups of 4 mice were tested at each dose.
†Mean total motor activity counts for 15 min ± standard error of the mean.
‡Comparison of drug treated to appropriate saline control group via Student's t-test.

20 times higher than its ED50 for antagonism of aggression, its activity was probably not due to nonspecific amphetamine-like stimulant properties.

d-Amphetamine (1.0 mg/kg, IP) significantly ($p<0.01$; Student's t-test) elevated motor activity in group-housed controls but failed to significantly ($p>0.05$) alter the activity of isolated mice. However, amphetamine significantly increased motor activity in both group-housed and isolated mice at 3 mg/kg, IP. Thus, isolated mice may be less sensitive to the motor stimulant properties of amphetamine as compared to group-housed mice.

GENERAL DISCUSSION AND CONCLUSIONS

TRH was found to be a selective (i.e., it antagonized aggression at doses which didn't produce neurological impairment) potent antagonist of isolation-induced aggression in male mice. Significant activity was still observed 3 hr postdrug administration although it was approximately 70 times less potent than at 30 min postdrug administration.

This activity of TRH appeared to be a direct affect on the CNS rather than related to the activity of TRH on the thyroid gland since neither T_3 nor any of the constituent amino acids of TRH had such activity in isolated mice. This conclusion is in agreement with the results of a previous study in isolated mice in which chronic treatment with thyroxine reduced the isolation time necessary to induce aggression whereas thiouracil had the opposite effect [31]. Thus, increased release of thyroid hormones would be expected to increase rather than decrease aggression.

TRH has been reported to produce significant increases in motor activity [3,21]; since such activity suggests an amphetamine-like effect, amphetamine was compared to TRH in isolated mice. d-Amphetamine sulfate failed to antagonize aggression over a wide range of doses. TRH failed to significantly alter spontaneous motor activity in either isolated or group-housed mice at doses at least 20 times TRH's ED50 for antagonism of aggression. Also, Breese and co-workers [3] demonstrated that TRH significantly reduced ethanol sleeping time whereas amphetamine significantly enhanced it in mice. Furthermore, electrophysiological studies indicated that TRH has a depressant action on neuronal activity in various regions of the CNS [9,20]. Thus, the antiaggressive activity of TRH was most likely not due to an amphetamine-like stimulant action.

The widespread occurrence of TRH in areas of the brain outside of the median eminence region of the hypothalamus would imply that it has a physiological role other than that of a releasing hormone [4, 11, 14]. In addition, Burt and Snyder [5] have demonstrated high-affinity receptor binding with TRH in rat brain membranes from various brain areas.

As previously noted, TRH exhibits many activities in both animals and man which appear unrelated to its affects on the thyroid gland. Many clinically effective antidepressant drugs (manuscript in preparation), as well as electroconvulsive shock treatment [13] selectively antagonized isolation-induced aggression in mice. Thus, the activity of TRH in this test may be related to its clinical antidepressant activity.

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