Pharmacology Biochemistry & Behavior, Vol. 13, pp. 137–139. Printed in the U.S.A.

TRH on Rat Conditioned Avoidance Behavior: Interaction with Brain Catecholamines[1]

SERGIO MORA, ANTONIA G. NASELLO AND LAIS FIESCHI

Laboratório de Medicina Experimental, Faculdade de Ciências Médicas de Santa Casa de São Paulo, São Paulo–Brazil

Received 4 October 1979

MORA, S., A. G. NASELLO AND L. FIESCHI. *TRH on rat conditioned avoidance behavior: Interaction with brain catecholamines*. PHARMAC. BIOCHEM. BEHAV. 13(1) 137–139, 1980.—TRH (10 μg) intracerebroventricularly injected improves the acquisition of a two-way avoidance conditioning. This effect is partially antagonized by pretreatment IP with α-methyltyrosine (60 mg/kg) or disulfiram (300 mg/kg). L-DOPA (100 mg/kg) administered IP 2 hr after α-MT partially restores the facilitatory effect of the hormone. The possible roles of brain catecholamines on the behavioral effect of TRH are analysed. Other tentative mechanisms of action are also discussed.

TRH Conditioned avoidance behavior Brain catecholamines α-Methyltyrosine Disulfiram L-DOPA

AN increasing amount of experimental and clinical evidence [20] that thyrotropin-releasing-hormone (TRH) may exert direct effects on the central nervous system, has been presented in the last few years. TRH reverses the barbiturate and ethanol narcosis in rodents [3,26]; potentiates both DOPA response in normal, hypophysectomized and thyroidectomized mice [24,25] and the behavioral changes induced by tryptophan [11] and 5-hydroxytryptophan [14]; and antagonizes the sedative and hypothermic actions of reserpine, chlorpromazine and diazepam [17]. In a previous paper, we reported that intracerebral administration of TRH in mice increases spontaneous motility, induces tremor, rotational and stereotyped behavior and counteracts the effects of some antipsychotic drugs [21].

The development of sensitive and specific immunoassays for quantifying TRH, in the central nervous system of the rat, has led to the unexpected discovery that as much as 80% of total brain TRH is in extrahypothalamic structures like thalamus, brain stem, cerebral cortex and cerebellum [16].

Although no significant increase or decrease in the content of any biogenic amine was observed following TRH, it has been established that dopamine (DA) content is raised 50% in mice prepared for the DOPA test [25], and that norepinephrine (NE) turnover can be accelerated in the cerebral cortex after the administration of TRH. Behavioral evidence also supports the hypothetic interaction between TRH and CNS catecholamines [5, 20, 21]. At present the importance of an undisturbed catecholamine system for the performance of conditioned avoidance behavior is well known [7]. Different papers have shown that activation of CNS catecholamine mechanisms is involved in the acquisition of conditioned avoidance response [8,23].

In view of the evidence presented above we decided to study the effect of TRH on the acquisition of a two-way avoidance conditioning and its interactons with α-methyltyrosine (α-MT) and disulfiram, whose effects on the biosynthesis of catecholamines are widely known [10,19].

METHOD

Subjects

Albino male rats of our colony (Wistar origin), 90–140 days old, 200–250 g body weight, were used. They were housed six to an appropriate cage in a temperature regulated room (23 ± 2°C) on a 12 hr light-dark cycle and they had food and water available ad lib. The rats were assigned randomly to the different control and experimental groups.

Active Avoidance Conditioning

As has been previously described [22], animals were training over 50 trials in a modified Warner shuttle-box. Each trial consisted of the presentation of a buzzer (conditioned stimulus) which after 5 sec overlapped with an electric shock (1.5 mA) to the grid on the floor (unconditioned stimulus), unless the animal crossed the midline barrier as a conditioned avoidance response. Intertrial interval was 30 sec.

Drugs. TRH (thyrotropin-releasing hormone or pyro-Glu-His-Pro-NH_2; Calbiochem) was dissolved in saline α-MT (DL-methyl-p-tyrosine; Calbiochem), L-DOPA (L-desoxyphenylalanine; Calbiochem) and disulfiram (Sigma Chem.) were suspended in a mixture of a phosphate buffer pH 6.5 and Tween 80 (10:0.2). All the drugs were adminis-

[1]This work was supported by Grants from FINEP-SEPLAN, Brazil, and the Servicio de Desarrollo Científico y Creación Artística de la Universidad de Chile, Chile.

TABLE 1

EFFECT OF TRH ON ACQUISITION OF A CONDITIONED RESPONSE. INFLUENCE OF PRETREATMENT WITH SALINE, α-MT (60 mg/kg), α-MT + L-DOPA (100 mg/kg) AND DISULFIRAM (300 mg/kg)

Treatment IP	ICV	n	Conditioned responses (mean ± SE)	Difference within groups
Saline	—	5	46.4 ± 7.3	$p>0.05$
TRH (5 mg/kg)	—	6	56.0 ± 6.7	
Saline	Saline	14	44.3 ± 4.2	$p<0.005$
Saline	TRH	9	67.6 ± 2.8	
α-MT	Saline	8	12.8 ± 5.3*	$p<0.05$
α-MT	TRH	10	28.6 ± 6.0*	
α-MT+L-DOPA	Saline	8	16.0 ± 5.9*	$p<0.05$
α-MT+L-DOPA	TRH	9	45.1 ± 8.7*	
Disulfiram	Saline	8	18.5 ± 3.5*	$p<0.05$
Disulfiram	TRH	6	45.0 ± 10.7*	

*Differences with their controls receiving saline IP are significant ($p<0.05$).
n=number of animals.

tered by intraperitoneal (IP) injections in volumes of 0.1 ml/100 g body weight, except TRH. This last drug was administered either IP like the others or intracerebroventricularly (ICV) through a cannula placed stereotaxically in the lateral ventricle.

Schedule of Drug Administration

Three hours before the beginning of the experiment the animals were injected IP with α-MT (60 mg/kg), disulfiram (300 mg/kg) or saline (0.1 ml/100 g). Half of the rats pretreated with α-MT received also L-DOPA (100 mg/kg) 2 hr later. Seven minutes before the training the animals treated above were injected ICV with TRH (10 μg in 5 μl of saline solution) or saline (5 μl) and were immediately placed in the conditioning box. Intraperitoneal effect of TRH was studied in an additional group of animals. They were injected with TRH (5 mg/kg) or saline 20 min before the training and placed in the shuttle-box 7 min before the beginning of the conditioning procedure.

Statistics

Differences between groups were assessed statistically by the Mann-Whitney U-test. They were considered to be significant when P was equal or less than 0.05.

RESULTS

Table 1 shows the results obtained. The percentage of conditioned avoidance responses is increased in the animals treated with TRH ICV, while the IP administration of the same substance does not have significant effect.

α-MT impaired the acquisition of the conditioned responses. This effect is not modified by the dose of L-DOPA used by us. The improving effect of TRH ICV is markedly reduced when this peptide is administered in both groups mentioned above. However, in the presence of L-DOPA the decrease in the number of avoidance responses is smaller.

Disulfiram also reduces the effect of TRH on the conditioned performance but this reduction is not so great as that observed when disulfiram is administered alone.

DISCUSSION

The present findings provide further evidence to support the hypothesis of a central action of the hypothalamic hormone TRH and its interaction with brain biogenic amines. Our results show that ICV administration of TRH improves the acquisition of a conditioned avoidance response. Nevertheless, as has been observed by other investigators [18], this effect is not evident after intraperitoneal injection of the hormone.

α-MT, injected prior to training, impairs acquisition of the conditioned avoidance response [2] and, in our experimental conditions, it reduces markedly, but does not abolish, the improving effect of TRH on this behavior.

α-MT decreases brain levels of DA and NE [27], as a consequence of inhibiting tyrosine hydroxylase, the rate limiting enzyme in catecholamine synthesis [19]. One could expect that L-DOPA antagonizes the behavioral depression induced by α-MT, but in our case the recovery was only partial, probably because of the dose used by us or the time of action. The effect of L-DOPA in reestablishing the performance level of rats pretreated with α-MT has been studied by others but their results are contradictory. While some have failed to observe a protective effect of L-DOPA [28] others have reported such an effect, either partial or total [9, 12, 23]. When TRH was administered in the animals pretreated with α-MT and L-DOPA there was a notable antagonism of the depressive effect of α-MT on acquisition. Also, pretreatment with disulfiram, a dopamine β-hydroxylase inhibitor that decreases the synthesis of NE [10], impairs conditioned behavior and antagonizes partially the facilitation induced by TRH.

Our findings suggest that TRH may play a role in learning processes, and that, at least in part, normal brain catecholaminergic levels, particularly NE, seem to be necessary for the behavioral effect of TRH. Several papers deal with the effects of TRH administration on brain catecholamines. It

has been shown, for example, that TRH has no significant effects on endogenous brain levels of NE or DA [4, 5, 15, 25]. Biochemical measurements have indicated that TRH administration results in a 20% increase in brain NE turnover [13] and this has been confirmed by histochemical studies [6]. Repeated exposure of rats to TRH produces a dose-time dependent increase in brain stem tyrosine hydroxylase activity, suggesting that this peptide increases the synthesis of brain catecholamines [1].

It was related that incubation of rat synaptosomes *in vitro* in the presence of TRH results in increased release of ^{3}H-DA and ^{3}H [13]. We cannot rule out out a possibility of an effect of TRH upon pre- or post-synaptic noradrenergic receptor, modifying their sensitivity to the endogenous neurotransmitter.

Further studies are obviously needed to elucidate the mechanism of action and functional significance of TRH, and other hypothalamic peptides, in the brain. The analysis of the influence of these substances in high cerebral functions other than merely pituitary control, and their interaction with CNS neurotransmitters and depressant drugs could aid in the understanding of the physiopathology of neurologic and mental disorders.

REFERENCES

1. Agarwal, R. A., R. B. Rastogi and R. L. Singhal. Changes in brain catecholamines and spontaneous locomotor activity in response to thyrotropin releasing hormone. *Res. Communs chem. pathol. Pharmac.* **15:** 743–752, 1976.
2. Alhenius, S. Inhibition of catecholamine synthesis and conditioned avoidance acquisition. *Pharmac. Biochem. Behav.* **1:** 347–350, 1973.
3. Breese, G. R., J. M. Cott, B. R. Cooper, A. J. Prange, Jr. and M. A. Lipton. Antagonisms of ethanol narcosis by thyrotropin releasing hormone. *Life Sci.* **14:** 1053–1063, 1974.
4. Breese, G. R., J. M. Cott, B. R. Cooper, A. J. Prange, M. A. Lipton and N. P. Plotnikoff. Effects of thyrotropin releasing hormone (TRH) on the actions of pentobarbital and other centrally acting drugs. *J. Pharmac. exp. Ther.* **193:** 11–22, 1975.
5. Cohn, M. L., M. Cohn and F. H. Taylor. Thyrotropin releasing factor (TRF) regulation of rotation in the non-lesioned rat. *Brain Res.* **96:** 134–137, 1975.
6. Constantinides, J., J. M. Gaillard, T. Horaguimian and R. Tissot. Enhancement of cerebral noradrenaline turnover by thyrotropin-releasing hormone: Evidence by fluorescence histochemistry. *Experientia* **30:** 1182, 1974.
7. Engel, J. and A. Carlsson. Catecholamines and behavior. In: *Current Developments in Psycopharmacology, Vol. 4.* New York: Spectrum Publ., 1977, pp. 1–32.
8. Fulginiti, A. S. and O. A. Orsingher. Effect of learning, amphetamine and nicotine on the level and synthesis of brain noradrenaline in rats. *Archs int. Pharmacodyn.* **190:** 291–298, 1971.
9. Fulginiti, S., V. A. Molina and O. A. Orsingher. Inhibition of catecholamines biosynthesis and memory processes. *Psychopharmacology* **51:** 65–69, 1976.
10. Goodman, L. S. and A. Gilman. *Pharmacological Basis of Therapeutics.* New York: Macmillan, 1975.
11. Green, A. R. and D. G. Grahame-Smith. TRH potentiates behavioral changes following increased brain 5-hydroxytryptamine acumulation in rats. *Nature* **251:** 524–526, 1974.
12. Hanson, L. C. F. Biochemical and behavioral effect of tyrosine hydroxylase inhibition. *Psychopharmacologia* **11:** 817, 1967.
13. Horst, W. D. and N. Spirt. A possible mechanism for the antidepressant activity of thyrotropin-releasing hormone. *Life Sci.* **15:** 1073–1082, 1974.
14. Huidobro-Toro, J. P., A. Scott de Carolis and V. G. Longo. Intensification of central catecholaminergic and serotoninergic processes by the hypothalamic factors MIF and TRH and by angiotensin II. *Pharmac. Biochem. Behav.* **3:** 235–242, 1975.
15. Keller, H. H., G. Bartholini and A. Pletscher. Enhancement of cerebral noradrenaline turnover by thyrotropin-releasing hormone. *Nature* **248:** 528–529, 1974.
16. Krulich, L., M. Quijada, J. E. Wheaton, P. Illner and S. M. McCann. Localization of hypophysiotropic neurohormones by assay of sections from various brain areas. *Fedn Proc.* **36:** 1953–1959, 1977.
17. Kruse, H. Thyrotropin-releasing hormone: interactions with chlorpromazine in mice, rats and rabbits. *J. Pharmac., Paris* **6:** 240–268, 1975.
18. Kulig, B. M. The effects of thyrotropin-releasing hormone on the behavior of rats pretreated with α-methyltyrosine. *Neuropharmacology* **14:** 489–492, 1975.
19. Levitt, M., S. Spector and S. Udenfriend. Elucidation of the rate-limiting step in norepinephrine bio-synthesis in the perfused guinea-pig heart. *J. Pharmac. exp. Ther.* **148:** 1–8, 1965.
20. Longo, V. G., S. Mora and A. Loizzo. Behavioral effects of the hypothalamic-releasing factors MIF and TRH. Laboratory data versus clinical results. In: *Psychotropic Action of Hormone,* edited by T. M. Itil, G. Laudahn and W. M. Herrmann. New York: Spectrum Publ., Inc., 1976.
21. Mora, S., A. Loizzo and V. G. Longo. Central effects of thyrotropin-releasing factor (TRF): Interaction with some antipsychotic drugs. *Pharmac. Biochem. Behav.* **4:** 279–282, 1976.
22. Nasello, A. G., C. A. Astrada and O. A. Ramirez. Effects on the acquisition of conditioned avoidance response and seizure threshold in the offspring of amphetamine treated gravid rats. *Psychopharmacologia* **40:** 25–31, 1974.
23. Orsingher, O. A. and S. Fulginite. Effects of alpha-methyltyrosine and adrenergic blocking agents on the facilitating action of amphetamine and nicotine on learning in rats. *Psychopharmacologia* **19:** 231–240, 1971.
24. Plotnikoff, N. P., A. J. Prange, Jr., G. R. Breese, M. S. Anderson and I. G. Wilson. Thyrotropin-releasing hormone on DOPA response in normal hypophysectomized, and thyroidectomized animals. In: *The Thyroid Axis, Drugs and Behavior,* edited by A. J. Prange, Jr. New York: Raven Press, 1974, pp. 103–113.
25. Plotnikoff, N. P., G. R. Breese and A. J. Prange, Jr. Thyrotropin-releasing hormone (TRH): DOPA potentiation and biogenic amines studies. *Pharmac. Biochem. Behav.* **3:** 665–670, 1975.
26. Prange, A. J., Jr., G. R. Breese, J. M. Cott, B. R. Martin, B. R. Cooper, I. C. Wilson and N. P. Plotnikoff. Thyrotropin-releasing hormone: Antagonism of pentobarbital in rodents. *Life Sci.* **14:** 447–455, 1974.
27. Rech, R. H., H. K. Borys and K. E. Moore. Alterations in behavior and brain catecholamine levels in rats pretreated with alpha-methyltyrosine. *J. Pharmac. exp. Ther.* **153:** 412–419, 1966.
28. Weissman, A. and B. K. Koe. Behavioral effects of L-methyltyrosine, an inhibitor of tyrosine hydroxylase. *Life Sci.* **4:** 1037–1048, 1965.