Pharmacology Biochemistry & Behavior, Vol. 4, pp. 279–282.

Central Effects of Thyrotropin-Releasing Factor (TRF): Interaction with Some Antipsychotic Drugs

S. MORA, A. LOIZZO AND V. G. LONGO

Laboratori di Chimica Terapeutica, Istituto Superiore di Sanità, Rome, Italy

(Received 14 July 1975)

MORA, S., A. LOIZZO AND V. G. LONGO. *Central effects of thyrotropin-releasing factor (TRF): interaction with some antipsychotic drugs.* PHARMAC. BIOCHEM. BEHAV. 4(3) 279–282, 1976. — The behavioral effects (tremors, tail erection, increased motility) of intracerebrally injected TRF are enhanced by pretreatment with chlorpromazine, reserpine and sulpiride; haloperidol does not exert appreciable effects. L-Dopa attenuates or abolishes the potentiation.

TRF L-Dopa Reserpine Sulpiride Haloperidol Chlorpromazine

THE recent isolation, characterization and synthesis of some hypothalamic releasing factors has led to numerous investigations on their biological activity. Several reports indicate that the thyrotropin-releasing factor (TRF), in addition to its effect on the pituitary-thyroid axis, has other sites and mechanisms of action which merit consideration. In mice and rats, TRF, administered intracerebrally in minute amounts (10–20 μg), stimulates motor activity, induces tremors and tail lifting (Straub reaction) [5,17]. The same excitatory syndrome could be obtained in rats and rabbits by parenteral administration of much higher doses (3–25 mg/kg) [5, 11, 16]. Moreover, TRF enhances the stimulant properties of L-Dopa and of 5-hydroxytryptophan in pargyline-pretreated mice and rats and antagonizes the depressant actions of pentobarbital and alcohol [4, 8, 15]. Interactions with other centrally acting drugs have also been studied. According to Breese *et al.* [4] the LD_{50} of pargyline, chlorpromazine, desipramine and phenelzine in mice is not affected by TRF pretreatment, while Kruse [10] reports that in mice and rabbits the acute toxicity of neuroleptics, antidepressants and of various other drugs (amphetamine, caffeine, scopolamine, strychnine) is substantially enhanced by TRF.

The present study was undertaken to evaluate the influence on the behavioral effects of TRF in pretreatment with chlorpromazine, reserpine, sulpiride, haloperidol. It is known that the spectrum of pharmacological activities of these compounds includes effects on the diencephalic centers, therefore an interaction with TRF would be expected.

METHOD

A total of 300 Swiss mice, weighing 20–30 g, were used. TRF was diluted in water and administered intracerebrally according to the method of Haley and McCormick [7]; the maximum amount of liquid injected was 10 μl. Chlorpromazine, sulpiride and haloperidol were injected intraperitoneally 1 or 2 hr before the administration of TRF; reserpine was injected 12 hr before TRF. The behavioral observations were carried out on groups of 5 animals placed in large Plexiglas cylinders 20 min before and immediately after treatment. Every group of TRF-treated animals had its own control group, injected with the same amount of solvent. The mice were observed every 10 min for as long as 2 hr for the presence of tremors, Straub tail phenomenon, motility, as well as reactivity to external stimuli (hand clap, touch) and aggressive and stereotypic behavior. In order to quantify the intensity of the response the various symptoms were evaluated as follows: tremor and hyperreactivity to external stimuli were scored +1 (moderate), +2 (marked) and +3 (very marked); Straub tail phenomenon was scored +1 (tail in extended position), +2 (tail in vertical positions) and +3 (tail extended horizontally in rostral position). Spontaneous motility was scored assigning 0 when spontaneous motility was absent, +1 to the animal showing slowness, +2 when the motility was apparently normal, +3 when stereotypic behavior (licking, self grooming, circling movements) was prevalent and +4 when increased motility, jumping, running, were present.

RESULTS

Behavioral Effects of Intracerebrally Injected TRF

Ten groups of 5 mice each were used to assess the effects of TRF, which was administered in doses of 2, 10 and 20

[1] Part of these results have been presented to the World Congress of Biological Psychiatry, Buenos Aires, Argentina, 24–28 Sept. 1974. S. Mora was on leave of absence from the Department of Experimental Medicine, East Area, Faculty of Medicine, University of Chile, Santiago, Chile. The technical assistance of Mr. Ezio Deodati is gratefully acknowledged.

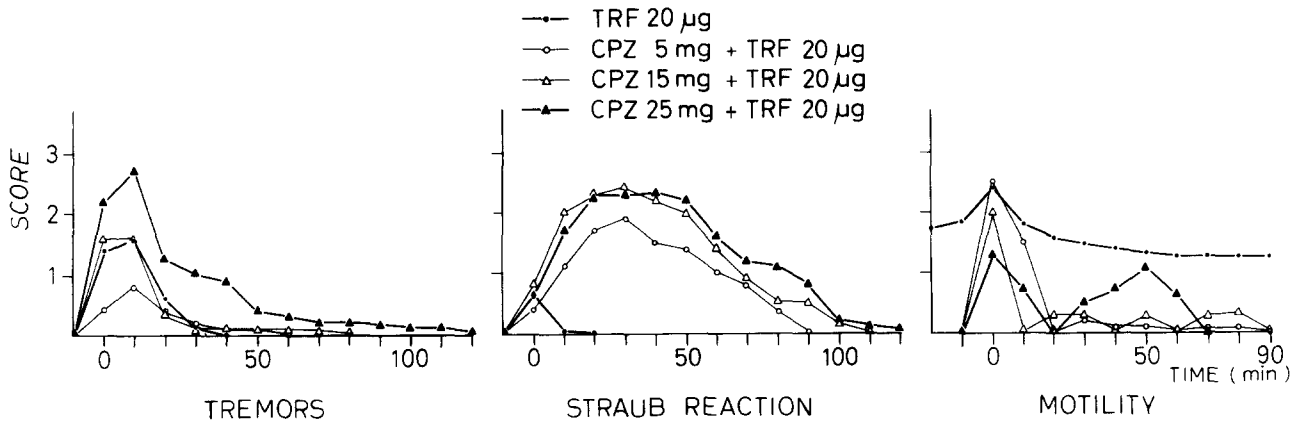

FIG. 1. Influence of chlorpromazine pretreatment on the behavioral effects of TRF (20 μg) in mice. In the graphs are charted the results obtained in the 3 behavioral tests used in the present experiments, see text for details. The tremors are slightly potentiated while the Straub response is markedly enhanced. The motor depression caused by chlorpromazine is reversed by TRF; in the mice treated with 25 mg/kg of chlorpromazine there is also a late phase of excitation.

μg/mouse. There was no noticeable difference in the response of mice to 10 and 20 μg. A few min after the administration, the animals exhibited a slight increase in motility with occasional running and jumping; moderate tremors and a Straub reaction were also present. The duration of these effects varied from 10 to 30 min. Two μg did not induce behavioral alterations different from those observed in mice injected with 10 μl of water intracerebrally. These animals reacted to the injection with a few jumps and running fits which never lasted longer than 2–3 min.

Effects of Pretreatment with Antipsychotic Drugs on the Behavioral Actions of TRF

Chlorpromazine. Chlorpromazine, in doses of 5, 15 and 25 mg/kg was administered respectively to 2 groups of 5 animals each. The animals in 2 additional groups were treated with saline. The chlorpromazine-treated animals developed a behavioral depression which was directly related to the dose. One hr later, 20 μg of TRF was administered. A few min later the animals treated with the highest dose of chlorpromazine exhibited a fine tremor of the paws and a +3 Straub reaction. Although the animals were incapable of coordinated movements, they started walking around the cage. Moreover, 40–60 min after TRF treatment, there was a return of spontaneous activity with occasional running and jumping. The groups treated with 5 and 15 mg/kg of chlorpromazine exhibited after TRF enhancement of Straub reaction and antagonism of motor depression, but not augmentation of tremors (Fig. 1).

The intracerebral administration of 10 μg of TRF to 2 groups of mice pretreated with chlorpromazine (25 mg/kg) induced ataxic walking, tremors and Straub tail phenomenon. The intensity and duration of the response was reduced when compared with manifestations induced by 20 μg of TRF. Also, the delayed enhancement of motility did not appear. TRF in the dose of 2 μg did not exert noticeable influence on the behavior of animals in 3 groups pretreated with 5, 15 and 25 mg/kg of chlorpromazine respectively, whose depression lasted as long as 6 hr. In this respect there was no difference with the chlorpromazine-pretreated animals who received an intracerebral injection of the solvent alone.

Sulpiride. Sulpiride, in doses of 10, 20 and 50 mg/kg was administered respectively to 2 groups of 5 mice each. The animals in 2 additional groups were treated with saline. No influence on gross behavior was exerted by these doses of sulpiride. One hr later, half of the groups received 20 μg of TRF and the other half was treated with the solvent. Of the various symptoms induced by TRF, only the tremors were enhanced by sulpiride. In the animals treated with the highest dose, the tremors lasted for 90 min (Fig. 2). The same experiment was performed using 10 μg of TRF; also with this dose potentiation of the tremors was present. Intracerebral administration of the solvent did not induce any symptom in the sulpiride-treated animals.

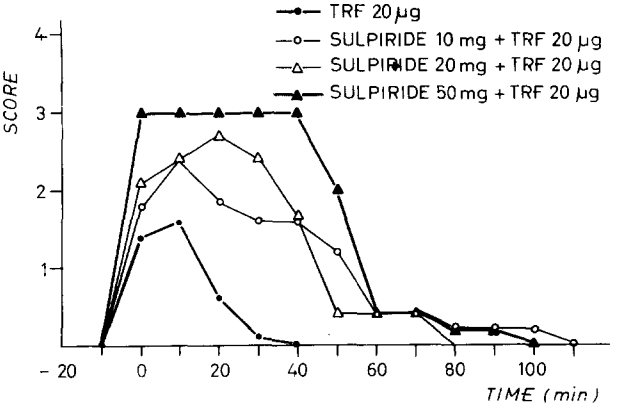

FIG. 2. Effects of various doses of sulpiride on the tremors induced by 20 μg of TRF. Potentiation of the tremors is already evident after pretreatment with 10 mg/kg of sulpiride and is maximal with 50 mg/kg.

Reserpine. Eight groups of 5 mice each were treated with 5 mg/kg of reserpine; 12 hrs later all the animals were inactive and unresponsive. TRF, in doses of 2, 10, and 20 μg, was injected in 2 groups of 5 mice each, while the last 2 groups received the solvent alone. In reserpine-pretreated animals, the intracerebral injection of 20 μg of TRF induced intense tremors lasting for about 2 hr and a short lasting return of motor activity was also observed (Fig. 3).

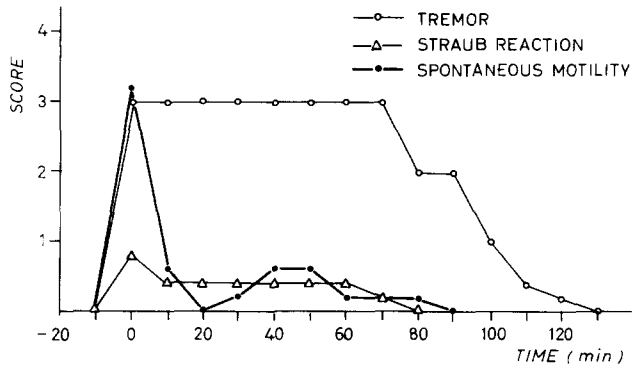

FIG. 3. Effects of reserpine on the behavioral symptoms induced by TRF. Intracerebral administration of 20 μg of TRF induces a short lasting reversal of the motor depression induced by reserpine and a marked potentiation of the tremors. For control response to TRF cf. Fig. 1.

Potentiation of tremors was also noticed in the animals treated with 10 and 2 μg of TRF and with the solvent alone. In this latter group, the tremors were less intense than those observed in TRF treated groups.

Haloperiodol. The intraperitoneal injection of 1, 2 and 5 mg/kg of haloperidol induced a behavioral depression which was quantitatively related to the dose. One or 2 hrs after the haloperidol pretreatment TRF (20 μg) was administered. The resulting effect of this combined treatment was an increase in the duration but not in the intensity of the tremors.

Effects of L-Dopa. In a subsequent experiment, the influence of L-Dopa on the potentiation of the behavioral effects of TRF exerted by chlorpromazine, reserpine and sulpiride was examined. Three groups of 5 animals each were treated respectively with chlorpromazine 25 mg/kg, reserpine 5 mg/kg and sulpiride 20 mg/kg. Thirty min before administration of TRF (20 μg) the animals received 100 mg/kg of L-Dopa intraperitoneally. L-Dopa abolished the motor excitation observed immediately after the administration of TRF in chlorpromazine pretreated mice and significantly reduced the Straub phenomenon. However, it had no effect on the tremors induced by TRF. The administration of L-Dopa to animals pretreated with sulpiride completely antagonized the tremors induced by TRF. The same antagonism, but less marked, was exerted by L-Dopa against the tremors induced by TRF in reserpine-treated mice.

DISCUSSION

The mechanism of the excitant action of TRF has not been determined; however, several data indicate that TRF could induce these effects independently from its pituitary influence. For example, pharmacological studies indicate that L-Dopa potentiation is induced by TRF also in hypophysectomized animals and radioimmunoassay methods have revealed that TRF is a normal constituent of extrahypothalamic brain tissue [18]. These findings suggest that TRF may have other functions in brain, besides that of regulating the release of hypophyseal hormones.

In the present experiments, the behavioral effects of intracerebrally injected TRF were found to be enhanced by pretreatment with some neuroleptic drugs: reserpine, chlorpromazine and sulpiride, while haloperidol did not exert any appreciable effect. This enhancement had a certain specificity in regard to the various symptoms induced by TRF; the Straub reaction was much more evident and lasted longer after chlorpromazine pretreatment, while tremors were potentiated and prolonged by sulpiride and reserpine.

The enhancement of the tremors cannot be attributed to a lowering in body temperature, which has been reported by Metcalf [13] in cats treated with intraventricular injections of TRF. In mice, 10 and 20 μg of TRF induce only a slight lowering (1–1.5°C) in rectal temperature. This lowering is not potentiated by pretreatment with sulpiride, haloperidol, or reserpine. The latter drug induces hypothermia in mice (7–8°C), which is reversed by the administration of TRF (unpublished observations from this laboratory).

Several hypotheses can be advanced to explain this potentiation, related to the sites and mechanism of action.

The spectrum of activities of antipsychotics includes effects on the diencephalic centers, where the maximal concentration of TRF has also been found [3,18]. The endocrinological effects observed both in animals and in men after the administration of chlorpromazine and reserpine have been attributed to an influence of these drugs on the monoaminergic neurons involved in the control of hormonal secretion from the pituitary. Clinically, sulpiride has antipsychotic properties with a strong thymoanaleptic component [2]; this drug has many pharmacological properties in common with the classical neuroleptics (amphetamine and apomorphine antagonism, inhibition of operant behavior, induction of catalepsy) and in addition has strong effects on the neuroendocrine system [12]. One is therefore tempted to relate this potentiation to an effect of these drugs on the neural hypothalamic systems related to release of the hypothalamic factors, which, in addition to pituitary stimulation, have other central actions. The fact that L-Dopa is able to abolish or to attenuate the potentiation exerted by these drugs can also be considered in favour of this hypothalamic mechanism. These experimental data have their counterpart in some results obtained in humans [14] which demonstrated that the inhibitory effects of chlorpromazine on the release of the prolactin inhibiting factor (PIF) can be reversed by administration of L-Dopa.

In our experimental conditions haloperidol exerted only a slight potentiating effect on TRF, consisting of a prolongation of the duration of the tremors. It should be noted in this connection that, among the drugs tested, haloperidol is the compound which as the strongest blocking effects on dopaminergic receptors. The potentiation might not be correlated with the amine-blocking properties of the neuroleptic drugs but rather with a more general influence on the hypothalamus and/or other brain centers. For instance, Schenkel-Hulliger *et al.* [16] and Breese *et al.* [4] have reported an enhancement of TRF induced tremors also in pentobarbital-pretreated animals.

With regard to the mechanism of action, biochemical and neurophysiological findings have been reported which indicate that TRF in high doses enhances the turnover of NE in the brain of rats [6,9]. This activation of the noradrenergic system might be the cause of the excitatory syndrome caused by TRF in various animal species. Among the effects of neuroleptic drugs on the biochemical systems connected with the monoaminergic neurons there is an increase in monoamines turnover and a block of the uptake

from the terminals (cfr. 1). The observed potentiation could be attributed to an additive effect on the neuronal activity of the monoamine system in the brain. The L-Dopa antagonism of the potentiation could be due to an inhibition of the enhanced turnover through a feedback process, originated by the presence in excess of one or more components of the biosynthetic chain.

REFERENCES

1. Andèn, N. E., A. Carlsson and J. Häggendal. Adrenergic mechanisms. *Ann. Rev. Pharmac.* **9:** 119–134, 1969.
2. Borenstein, P., C. Champion, Ph. Cujo, F. Gekière, C. Olivenstein and P. Kramarz. Un psychotrope original: le sulpiride. *Sem. Hop.* **45:** 1301–1314, 1969.
3. Brownstein, M. J., M. Palkovits, J. M. Saavedra, R. M. Bassiri and R. D. Utiger. Thyrotropin-releasing hormone in specific nuclei of rat brain. *Science* **185:** 267–269, 1974.
4. Breese, G. R., B. R. Cooper, A. J. Prange Jr, J. M. Cott and M. A. Lipton. Interactions of thyrotropin-releasing hormone with centrally acting drugs. In: *The Thyroid Axis, Drugs and Behavior,* edited by A. J. Prange, Jr. New York: Raven Press, 1974, pp. 115–127.
5. Breese, G. R., J. M. Cott, B. R. Cooper, A. J. Prange Jr. and M. A. Lipton. Antagonism of ethanol narcosis by thyrotropin-releasing factor. *Life Sci.* **14:** 1053–1063, 1974.
6. Constantinidis, J., F. Geissbühler, J. M. Gaillard, Th. Hovaguiminian and R. Tissot. Enhancement of brain noradrenaline turnover by thyrotropin-releasing hormone: evidence by fluorescence histochemistry. *Experientia* **30:** 1182–1183, 1974.
7. Haley, T. J. and W. G. McCormick. Pharmacological effects produced by intracerebral injection of drugs in the conscious mice. *Br. J. Pharmac.* **12:** 12–15, 1957.
8. Huidobro Toro, J. P., A. Scotti de Carolis and V. G. Longo. Intensification of central catecholaminergic and serotonergic processes by the hypothalamic factors MIF and TRF and by angiotensin II. *Pharmac. Biochem. Behav.* **3:** 235–242, 1975.
9. Keller, H. H., G. Bartholini and A. Pletscher. Enhancement of cerebral noradrenaline turnover by thyrotropin-releasing hormone. *Nature* **248:** 528–529, 1974.
10. Kruse, H. Neurotropic effects of thyrotropin-releasing factor. *Arch. Pharmac.* **282:** (Suppl.): R51, 1974.
11. Kruse, H. Thyrotropin-releasing hormone: interaction with chlorpromazine in mice, rats and rabbits. *J. Pharmac. (Paris)* **6:** 249–268, 1975.
12. Lanza, M., D. Picard and N. Carlon. Effets du sulpiride sur le tractus génital, la glande mammaire et l'hypophyse antérieure de la ratte. *C. r. Soc. Biol.* **165:** 1363–1368, 1971.
13. Metcalf, G. TRH: a possible mediator of thermoregulation. *Nature* **252:** 310–311, 1974.
14. Noel, G. L., H. K. Suh and A. G. Frantz. L-Dopa suppression of TRH-stimulated prolactin release in man. *J. Clin. Endocrinol. Metab.* **36:** 1255–1258, 1973.
15. Plotnikoff, N. P., A. J. Prange Jr., G. R. Breese, M. S. Anderson and I. C. Wilson. Thyrotropin-releasing hormone: enhancement of l-Dopa activity by a hypothalamic hormone. *Science* **178:** 417–418, 1972.
16. Schenkel-Hulliger, L., W. P. Koella, A. Hartmann and L. Maître. Tremorogenic effect of thyrotropin-releasing hormone in rats. *Experientia* **30:** 1168–1170, 1974.
17. Segal, D. S. and A. J. Mandell. Differential behavioral effects of hypothalamic polypeptides. In: *The Thyroid Axis, Drugs and Behavior,* edited by A. J. Prange, Jr. New York: Raven Press, 1974, pp. 129–133.
18. Winokur, A. and R. D. Utiger. Thyrotropin-releasing hormone: regional distribution in rat brain. *Science* **185:** 265–267, 1974.