Published in final edited form as:
J Pharmacol Exp Ther. 1976 March ; 196(3): 594–604.

INVESTIGATIONS INTO THE MECHANISM OF REDUCTION OF ETHANOL SLEEP BY THYROTROPIN - RELEASING HORMONE (TRH)[1]

JERRY M. COTT, **GEORGE R. BREESE**, **BARRETT R. COOPER**, **T. STEVEN BARLOW**, and **ARTHUR J. PRANGE JR.**
Departments of Psychiatry and Pharmacology, Biological Sciences Research Center, Child Development Institute, University of North Carolina, Chapel Hill, North Carolina

Abstract

Thyrotropin-releasing hormone (TRH), administered intraperitoneally, was found to antagonize ethanol-induced sleep and hypothermia in mice without affecting brain ethanol content. This reduction of the actions of ethanol was also apparent after oral or intracisternal administration of TRH. In addition, TRH reduced ethanol-induced sleep in rats, hamsters, gerbils and guinea pigs. Evidence that the pituitary-thyroid axis is not necessary for the effects of TRH was provided by observations that hypophysectomy did not reduce TRH antagonism of ethanol narcosis and findings that neither triiodothyronine nor thyrotropin mimicked its action. Certain analogs of TRH, which have little effect on the pituitary, were also found to antagonize ethanol-induced sleep and hypothermia. Pretreatment with the antiadrenergic drugs, α-methyltyrosine, phentolamine and propranolol did pot antagonize the ability of TRH to reduce sleep induced by ethanol. However, after intracisternal administration of atropine methyl nitrate, TRH no longer caused a significant reduction of sleep, even though TRH antagonism of the ethanol-induced hypothermia was still apparent. In contrast, central administration of other anticholinergic drugs, such as *d*-tubocurarine and hexamethonium, reduced ethanol-induced sleep and this effect was additive with TRH. Carbachol also reduced ethanol sleeping time and this effect was also blocked by atropine methyl nitrate. The antagonism of ethanol-induced sleep by dibutyryl cyclic adenosine 3′,5′-monophosphate was significantly *reduced* but not *blocked* by atropine methyl nitrate. Results provide evidence that TRH has a direct extrapituitary action on brain and that both TRH and ethanol may interact with central cholinergic systems.

Recently much attention has been given to the possibility that thyrotropin-releasing hormone (TRH) may possess extrapituitary actions on brain in addition to its acknowledged endocrine function to release thyrotropin and prolactin from the anterior pituitary (Bowers *et al.*, 1971). Evidence for a central effect of TRH was first provided by the finding that TRH potentiated L-dopa-induced excitation in pargyline pretreated animals following hypophysectomy (Plotnikoff *et al.*, 1972). Later, it was discovered that TRH reduced the sedation and hypothermia produced by pentobarbital (Prange *et al.*, 1974; Breese *et al.*, 1975) and ethanol (Breese *et al.*, 1974a,b).

[1]This work was supported by U.S. Public Health Service Grants MH-16522, AA-02334, HD-03110 and MH-15631, and a grant from the North Carolina Alcoholism Research Authority. G.R.B. is supported by a Career Development Award (MH-00013) and A.J.P.Jr., by a Career Scientist Award (MH-22536).

Send reprint requests to: Dr. George R. Breese, Department of Psychiatry and Pharmacology, 224 Biological Sciences Research Center, University of North Carolina School of Medicine, Chapel Hill, NC. 27514..

The present experiments were conducted to explore further the reversal of ethanol sedation produced by TRH. This was of particular interest since a greater understanding of this action of TRH, to antagonize ethanol and its possible mechanisms, could have implications for a better understanding of the pharmacology of ethanol, as well as for the central effect of TRH.

Methods

General

Male Swiss-Webster mice (19–27 g) obtained from Flow Laboratory (Dublin, Va.) were treated intraperitoneally with doses of ethanol (25% v/v) ranging from 4.3 to 4.7 g/kg. Doses used were chosen to maintain control sleeping time within the general range of 70 to 100 minutes. Sleeping time was measured as the time from which righting reflex was lost after ethanol administration until it was regained. Three rightings of the mouse within 60 seconds were considered to constitute return of righting reflex. The ability of TRH (pyroGlu-His-Pro-NH_2; Abbott Laboratories, North Chicago, Ill.) to antagonize ethanol was also studied in hypophysectomized mice (Charles River Breeding Laboratories, Wilmington, Mass.), Sprague Dawley rats (Zivic-Miller Laboratories, Pittsburgh, Pa.), Mongolian gerbils (Tumble farm, West Brookfield, Mass.), Syrian hamsters (Engle Laboratory, Farmersberg, Ind.), and Hartley guinea pigs (Camm Research Institute, Inc., Wayne, N.J.). All animals were maintained on a 12-hour light-dark cycle. Experiments were performed between 1:00 and 5:00 P.M. Volume of all intraperitoneal injections other than ethanol corresponded to 1% of body weight in mice, gerbils and hamsters and 0.1% body weight in rats and guinea pigs. Rectal temperatures were obtained in some experiments with a thermistor probe from Yellow Springs Instrument Company (Yellow Springs, Ohio). Only those time periods which previously had been found to correspond to the maximum period of hypothermia after ethanol were observed.

Drug treatments

In one experiment, mice were treated intraperitoneally with bovine thyrotropin (Armour Pharmaceutical Co., Chicago, Ill.) or L-triiodo-thyronine (Smith Kline and French Laboratories, Philadelphia, Pa.). In other experiments, mice were injected intracisternally with various analogs of TRH and other peptides including: pyroGlu-Lys-Pro-NH_2 (lysine TRH), pyroGlu-His-Pro (deamidated TRH), pyroGlu-2-4-diiodo-His-Pro-NH_2 (diiodo TRH), pyroGlu-(pyrazolyl-3) Ala-Pro-NH_2 (pyrazolyl TRH), pyroGlu-His-OMe (OMeDP), pyroGlu-His-Pro-βAla-TRH (β-alanine TRH) (Hoffmann La Roche, Nutley, N.J.); Pro-Leu-Gly-NH_2[2] (Abbott Laboratories); somatotropin release inhibiting factor (SRIF, Salk Institute, San Diego, Calif.); leutinizing hormone releasing hormone (LHRH) and substance P (Beckman, Palo Alto, Ca.). The effect of atropine methyl nitrate (Sigma Chemical Company, St. Louis, Mo.) on the action of TRH was examined after intraperitoneal and intracisternal administration . Hexamethonium bromide (Sigma), *d*-tubocurarine chloride (Abbott) or gallamine triethiodide (Davis and Geck, Danburg, Conn.), as well as the cholinomimetic carbachol (Sigma), were tested for their ability to alter TRH antagonism of ethanol. Other compounds examined include theophylline, dibutyryl adenosine 3′,5′-monophosphate (dibutyryl cyclic AMP), L-norepinephrine hydrochloride, diisopropylfluorophosphate (Sigma); eserine salicylate (Schwarz-Mann, Orangeburg, N.Y.); *dl*-propranolol hydrochloride (Ayerst Laboratories, New York, N.Y.), phentolamine hydrochloride, xylometazoline hydrochloride (Ciba Pharmaceutical Company, Summitt, N.J.) and *d*-amphetamine sulfate (Smith Kline and French). The effects of TRH on ethanol

[2]This tripeptide is sometimes designated melanocyte stimulating hormone release inhibiting factor (MIF). It exerts some but not all of the properties of the native substance (Vale *et al.*, 1973).

narcosis were also examined after α-methyl-tyrosine methyl ester hydrochloride (Regis Chemical Company, Chicago, Ill.

In some experiments involving intracisternal administration of drugs, it was necessary to determine doses that did not produce overt toxicity (*e.g.*, respiratory depression, convulsion or death). Although this procedure involved a degree of trial and error, it was possible in each case to determine doses for a given compound which did not show toxicity but retained actions consistent with its pharmacological activity. All drugs given intracisternally were administered in 10 μl of isotonic saline 10 minutes after ethanol. Drug weights are expressed as salts.

Biochemical procedures

Brain ethanol concentration was measured by the diffusion method described by Sunshine and Nenad (1953).

Statistics

Group comparisons were made with two-tailed Student's or Dunnett's *t* test as appropriate. A $P < .05$ was considered significant. Correlational analyses were performed by multiple regression.

Results

Effect of TRH on ethanol-induced sleep and hypothermia

In agreement with previous findings (Breese *et al.*, 1974a, b), TRH significantly reduced ethanol-induced sleep (table 1). Induction of sleep after ethanol occurred within 80 to 100 seconds and was not significantly altered by simultaneous treatment with TRH. After administration of TRH, a characteristic fine tremor of the forelimbs, increased respiration, increased muscle tone, piloerection, lacrimation and squinting of the eyes were evident within 2 minutes. When various doses of TRH findings (0.01–20 μg) were administered intracisternally, it was possible to reduce ethanol sleeping time with TRH in a dose-related fashion (table 1). Although the reason is not obvious, intracisternal injection of saline caused an increase in sleep when compared with an intraperitoneal injection of saline. A dose-response relationship was not apparent when TRH was given intraperitoneally or orally (see table 1). A 1 to 2°C reduction of ethanol-induced hypothermia was observed after TRH regardless of the route of administration ($P < .01$; data not shown)

In addition, TRH produced a significant antagonism of ethanol-induced sleep and hypothermia ($P < .01$) if administered 15 minutes before or 15 minutes after ethanol, but did not awaken mice when injected 30 minutes or longer before ethanol. It was also observed that TRH would reduce ethanol sleeping time in several species other than mice (table 2).

Relationship of TRH antagonism of ethanol-induced sleeping time to pituitary function

In order to test the possibility that the effect of TRH on ethanol sleeping time might be due to an action on the pituitary, the antagonism of ethanol by TRH was examined in hypophysectomized mice. As shown in table 3, hypophysectomy did not alter the actions of TRH. While the effect of TRH on sleeping time is obvious, interpretation of the effects of TRH on temperature is complicated by the fact that some mice awoke from sleep before rectal ternperature was measured. Nevertheless, it was found that intraperitoneal injection of doses of thyrotropin (up to 10 mg/kg) and L-triiodothyronine (up to 1 mg/kg), far in excess of the amount that could be released physiologically (Tata, 1964), did not reduce sleeping time or otherwise resemble the actions of TRH (table 3). Therefore, it is concluded that the pituitary and/or the thyroid are not essential for these actions of TRH.

Effect of intracisternally administered TRH derivatives and other hypothalamic peptides on ethanol-induced sleep and hypothermia

Following the observation that TRH would alter ethanol-induced sleep and hypothermia, several TRH derivatives and other peptides isolated from brain were administered intracisternally to determine whether they might also affect the soporific action of ethanol. As shown in table 4, several structural analogs of TRH were found to possess activity comparable to TRH. These included pyrazolyl TRH, lysine TRH, diiodo TRH and *β*-alanine TRH. Although deamidated TRH did not reduce ethanol-induced sleep, this metabolite of TRH (Nair *et al.*, 1971) did antagonize ethanol-induced hypothermia. Certain other peptides isolated from brain such as MIF, SRIF and LHRH did not alter ethanol-induced sleep, whereas substance P significantly shortened it. A dipeptide (pyroGlu-His-OMe) which has been found to inhibit TRH metabolism (Vale *et al.*, 1971), significantly potentiated the analeptic activity of TRH (table 4).

Effect of TRH on ethanol metabolism

Since TRH might reduce sleeping time by increasing the metabolism of ethanol, this possibility was examined (table 5). Brain levels of ethanol were determined at several intervals after ethanol administration with and without TRH. Although there was a tendency for TRH-treated mice to have lower brain ethanol levels, no significant difference between the two groups was observed.

Relationship of TRH with noradrenergic mechanisms

Earlier reports (Keller *et al.*, 1974; Reigle *et al.*, 1974; Horst and Spirt, 1974; Breese *et al.*, 1975; Constantinidis *et al.*, 1974) have indicated that TRH may increase the activity of noradrenergic neurons in brain. Recently, L-norepinephrine (30 *μ*g) has been shown to reduce pentobarbital sleeping time, an effect blocked by phentolamine (2 *μ*g) (Breese *et al.*, 1975). The possibility that TRH might be acting through a noradrenergic mechanism to antagonize ethanol-induced sleep was explored by comparing the effects of TRH with intracisternally administered L-norepinephrine (0.2 to 30 *μ*g). In other experiments, the *alpha* adrenergic agonist xylometazoline (0.2–20 *μ*g) (Mujic *et al.*, 1965) and the central nervous system (CNS) stimulant *d*-amphetarnine (4–100 *μ*g) were administered intracisternally. As seen in table 6, intracisternal administration of these compounds caused either no effect or an increase in sleeping time, and their effects on rectal temperature were different from that of TRH. They also failed to produce the tremor and increased respiratory rate that was observed after TRH treatment.

Other experiments were concerned with the effects of various agents which are believed to antagonize noradrenergic function. In this work, neither pretreatment with 300 mg/kg of *α*-methyltyrosine nor intracisternal pretreatment with phentolamine (2 *μ*g) or propranolol (10–35 *μ*g) antagonized the efficacy of TRH to reduce sleeping time produced by ethanol. In fact *α*-methyltyrosine and phentolamine appear to potentiate the sleep reduction caused by TRH. Although 35 *μ*g of propranolol and 2 *μ*g of phentolamine significantly reduce the antagonism of ethanol-induced hypothermia by TRH, they do not block this effect completely (see legend, table 6).

Examination of possible cholinergic mechanisms in the TRH antagonism of ethanol anesthesia

As can be seen in table 7, intracisternal administration of atropine methyl nitrate prior to the intraperitoneal or intracisternal injection of TRH caused a significant antagonism of the analeptic activity of TRH. In contrast to the antagonism of TRH effects on ethanol-induced sleep, elevation of rectal temperature by TRH was not blocked by intracisternally-

administered atropine methyl nitrate. Furthermore, intraperitoneal administration of 3 mg/kg of atropine methyl nitrate was not found to affect the reduction of ethanol-induced sleep produced by TRH, providing evidence that the effects of atropine methyl nitrate observed are due to an action on the central nervous system (38.3 ± 5.7% reduction of sleep by TRH in the saline pretreatment group compared to 43.9 ± 10.1% reduction in the atropine methyl nitrate pretreatment group; $P > .1$).

In order to investigate the possibility that other cholinergic blocking agents might influence the actions of TRH, several drugs known to be effective nicotinic receptor blockers in the peripheral nervous system at both the neuromuscular junction and autonomic ganglia were administered intracisternally. As can be seen in table 8, *d*-tubocurarine, gallamine and hexamethonium reduced ethanol-induced sleep. The doses reported are those which were found to be the most efficacious in preliminary trials and correlate quite well with potencies reported by Beleslin *et al.* (1974a,b) for CNS excitation in cats. Although the effects of these drugs somewhat resembled the actions of TRH in that they caused increased respiration, piloerection, eye blinking and scratching movements, no change in the ethanol-induced hypothermia was observed ($P > .1$; data not shown). When these compounds were administered with TRH, their analeptic effects were additive, producing a marked reduction of ethanol-induced sleep. After atropine methyl nitrate, the action of hexamethonium against ethanol-induced sleep was significantly reduced, whereas that of *d*-tubocurarine and gallamine was not (table 8).

Carbachol also reduced ethanol sleeping time and this effect was antagonized by centrally-administered atropine methyl nitrate (table 8). Paradoxically, when this agent was administered with TRH, the ability of TRH to antagonize ethanol was reduced. Intracisternal administration of the cholinesterase inhibitors, physostigmine (3 μg) and diisopropylfluorophosphate (1 μg), did not significantly affect ethanol-induced sleeping time ($P > .1$) or the efficacy of TRH to reverse ethanol-induced sleep ($P > .1$; data not shown).

Possible role of cyclic AMP in the action of TRH

Since the preliminary report by Cohn *et al.* (1973) that intraventricular dibutyryl cyclic AMP reduced ethanol sleeping time in rats, studies were undertaken to determine whether dibutyryl cyclic AMP would either resemble TRH or alter the effects of TRH against ethanol and if phosphodiesterase inhibition would influence the actions of TRH. As shown in table 9, theophylline did not significantly affect ethanol sleeping time nor did it enhance the action of TRH to antagonize ethanol-induced sleep. Dibutyryl cyclic AMP by itself reduced the sleep but did not antagonize the hypothermia induced by ethanol. When TRH was administered intraperitoneally in combination with intracisternally-injected dibutyryl cyclic AMP, an additive effect was not apparent (table 9). While pretreatment with 10 μg of atropine methyl nitrate significantly reduced the effect of this nucleotide, neither dose of the antimuscarinic agent prevented the reduction of ethanol-induced sleep by dibutyryl cyclic AMP as was the case with TRH (table 9).

Discussion

These results confirm earlier reports (Breese *et al.*, 1974a, b) that TRH produces a marked reduction of ethanol sleeping time and hypothermia, and suggest that this effect is due to an action of TRH directly on the CNS. Support for this view was provided by the findings that TRH is effective in hypophysectomized mice and that neither thyrotropin nor L-triiodothyronine resembled TRH in this analeptic activity. Furthermore, analogs of TRH found to antagonize ethanol provided no correlations between ability to release TSH (Vale *et al.*, 1973) and activity to reduce ethanol sleeping time and hypothermia. In the present work, it was found that the onset of action of TRH with regard to increased muscle tone and tremor

in ethanol-treated mice is quite rapid and of short duration which is in accord with the short half-life of TRH in plasma (May and Donabedian, 1973). Although the action of TRH against ethanol-induced sleep can be produced in other laboratory animals, it was noted that TRH failed to reduce ethanol-induced hypothermia in rats and guinea pigs while effectively reducing sleeping time. This latter finding and that of Prange *et al.* (1975), who showed that TRH is able to reduce pentobarbital sleeping time even when warm ambient temperatures prevent the hypnotic-induced hypothermia, suggests that the TRH reduction of ethanol-induced sleep does not depend upon an antagonism of the drug-induced hypothermia.

In mice, TRH was found to produce a dose-dependent reduction of ethanol sleeping time when administered intracisternally. Although TRH was effective after oral or intraperitoneal administration, a dose-response relationship was not apparent. The absence of a dose-response relationship to TRH has been reported previously in pentobarbital-treated mice (Breese *et al.*, 1975). Perhaps studies of the metabolism, distribution and, possibly, uptake systems for entrance of TRH into brain will resolve the lack of a dose-response relationship after peripheral administration. Although the doses used in the present study are beyond the physiological range to activate the pituitary, they are far below doses of TRH which cause death. For example, Piva and Steiner (1971) reported that the LD5O of TRH after intravenous administration to rats was 2500 mg/kg. In mice, intraperitoneal administration of doses as high as 2500 mg/kg are not lethal (unpublished data).

In regard to possible involvement of neurotransmitter systems in the actions of TRH, current evidence indicates that TRH produces a small but consistent increase in brain norepinephrine turnover (Keller *et al.*, 1974; Horst and Spirt, 1974; Breese *et al.*, 1975; Reigle *et al.*, 1974; Constantinidis *et al.*, 1974). However, in spite of this action of TRH, it does not appear likely that activation of a noradrenergic system is responsible for its antagonism of ethanol sleeping time. This conclusion is based upon the finding that the adrenergic agonists norepinephrine, xylometazoline and amphetamine administered into brain did not reduce sleep or otherwise resemble the actions of TRH and that neither α-methyltyrosine nor the adrenergic blocking drugs, propranolol and phentolamine, reduced the antagonism of ethanol by TRH.

The observation that intracisternal injection of atropine antagonized the effect of TRH to shorten pentobarbital-induced sleep (Breese *et al.*, 1975) prompted exploration of the interactions of TRH with various cholinergic and anticholinergic drugs in ethanol-treated mice. As in previous experiments with pentobarbital, atropine methyl nitrate was found to antagonize the ability of TRH to reduce ethanol-induced sleep. This observation prompted further work with other anticholinergic drugs to determine whether they might also antagonize effects of TRH. Intracisternal injection of compounds which block nicotinic receptors was found to shorten ethanol-induced sleep. In contrast to the action of atropine, these drugs appeared additive with TRH in their antagonism of ethanol sleeping time. However, it is not clear at this time whether the effects of these drugs can be attributed to their nicotinic blocking capacity, because the relative potencies of these agents to reduce ethanol narcosis are not correlated with either neuromuscular or ganglionic blocking activity (Volle and Koelle, 1970; Koelle, 1970). Rather, there is close correlation to both absolute and relative potency to cause CNS stimulation in the cat (Beleslin *et al.*, 1974a, b). In addition, a cholinergic agonist, carbachol, when administered intracisternally, was also found to reduce ethanol sleeping time. However, when carbachol was combined with TRH, rather than observing an additive or synergistic effect, the combination did not reduce sleeping time at all. It was also observed that, like the observation with TRH, atropine methyl nitrate would antagonize the effects of carbachol as well as the action of hexamethonium against ethanol-induced sleep. Although we could conclude that cholinergic systems in brain play a role in ethanol narcosis as have others (Erickson and Graham, 1973;

Phillis and Jhamandas, 1971; Kalant and Grose, 1967), the data do not permit a unified hypothesis on the nature of the interaction of cholinergic systems with ethanol. These data might also suggest that TRH interacts with cholinergic mechanisms in brain, but any interpretation at the present time would seem highly speculative due to the complex nature of the results.

Volicer and Gold (1973) suggested that cyclic AMP may play a role in the actions of ethanol, based on the finding that ethanol reduced cyclic AMP content in brain. In accord with this view, dibutyryl cyclic AMP was found to antagonize the sleep produced by ethanol in the rat (Cohn *et al.*, 1973). In the present work, intracisternally-administered dibutyryl cyclic AMP was found to reduce ethanol-induced sleep in mice. Since TRH and cyclic AMP both reduce ethanol sleeping time, it seemed possible that cyclic AMP might be mediating the effects of TRH. However, the actions of dibutyryl cyclic AMP were neither similar to nor additive with TRH and unlike TRH the effect of dibutyryl cyclic AMP, although reduced, was not blocked by atropine methyl nitrate. Thus, at present, it would be difficult to understand how endogenous cyclic AMP could be directly involved in the antagonism of ethanol-induced sleep by TRH, although this may be an interesting area for future research.

In conclusion, TRH appears to have the ability to reduce ethanol narcosis *via* an extrapituitary action on the CNS. Indeed, TRH is normally located in many regions of brain other than hypothalamus (Jackson and Reichlin, 1974a; Winokur and Utiger, 1974) and is present in amphibians and gastropods, where it appears to have no thyroid function (Grimm-Jorgensen and McKelvy, 1974; Taurog *et al.*, 1974; Jackson and Reichlin, 1974b; Grimm-Jorgensen *et al.*, 1975). For these reasons and for its behavioral effects, TRH has been proposed to act as a neurotransmitter (Breese *et al.*, 1974a; Jackson and Reichlin, 1974a; Winokur and Utiger, 1974). Data in this manuscript would not be inconsistent with this view.

Acknowledgments

Dr. Jean Rivier, of the Salk Institute, provided our laboratory with linear SRIF (somatostatin). Pyrazolyl TRH, *β*-alanine TRH, diiodoTRH, lysine TRH and deamidated TRH were gifts of Hoffmann-La Roche, Inc., Nutley, N.J. The authors acknowledge the generous donations of MIF and TRH from Nicholas P. Plotnikoff at Abbott Laboratories, North Chicago, Ill., and the excellent assistance of Joseph Farmer, Marcine Kinkead, Susan Hollister and Edna Edwards.

References

Beleslin DB, Grbović L, Radmanović BZ. The pharmacology of gross behavioral effects of cholinomimetic substances injected into the cerebral ventricles of unanesthesized cats: Evidence for central muscarinic mediation. Neuropharmacology 1974a;13:1163–1169. [PubMed: 4457763]

Beleslin DB, Samardzic R, Malobabic ZS. An analysis of the gross behavioral changes induced by tetraethylammonium and a comparison with other substances affecting the autonomic and central nervous system after intraventricular injections to conscious cats. Neuropharmacology 1974b; 13:1171–1177. [PubMed: 4156394]

Bowers CY, Friesen HG, Hwang P, Guyda HJ, Folkers K. Prolactin and thyrotropin release in man by synthetic pyroglutamyl-histidylprolinamide. Biochem. Biophys. Res. Commun 1971;45:1003–1041.

Breese, GR.; Cooper, BR.; Prange, AJ.; Cott, JM.; Lipton, MA. Interactions of thyrotropin-releasing hormone with centrally acting drugs. In: Prange, AJ., editor. The Thyroid Axis, Drugs and Behavior. Raven Press; New York: 1974a. p. 115-127.

Breese GR, Cott JM, Cooper BR, Prange AJ, Lipton MA. Antagonism of ethanol narcosis by thyrotropin releasing hormone. Life Sci 1974b;14:1053–1063. [PubMed: 4207201]

Breese GR, Cott JM, Cooper BR, Prange AJ, Liption MA, Plotnikoff NP. Effects of thyrotropin releasing hormone (TRH) on the actions of pentobarbital and other centrally acting drugs. J. Pharmacol. Exp. Ther 1975;193:11–22. [PubMed: 805836]

Cohn ML, Kraynack B, Cohn M, Scattaregia F. Interaction of cyclic AMP with neuropharmacologic depressant agents. Fed. Proc 1973;32:680.

Constantinidis J, Geissbuhler F, Gailland JM, Hovaguimian TH, Tissot R. Enhancement of cerebral noradrenaline turnover by thyrotropin-releasing hormone: Evidence by fluorescence histochemistry. Experientia (Basel) 1974;30:1182–1183. [PubMed: 4215671]

Erickson CK, Graham DT. Alteration of cortical and reticular acetylcholine release by ethanol *in vivo*. J. Pharmacol. Exp. Ther 1973;185:583–593. [PubMed: 4712652]

Grimm-Jorgensen Y, McKelvy JF. Biosynthesis of thyrotropin releasing factor by newt (*Triturus viridescens*) brain *in vitro*. Isolation and characterization of thyrotropin releasing factor. J. Neurochem 1974;23:471–478. [PubMed: 4214528]

Grimm-Jorgensen Y, McKelvy JF, Jackson IMD. Immunoreactive thyrotropin releasing factor in gastropod circumoesophageal ganglia. Nature (London) 1975;254:620. [PubMed: 165422]

Horst WD, Spirt N. A possible mechanism for the antidepressant activity of thyrotropin releasing hormone. Life Sci 1974;15:1073–1082. [PubMed: 4157277]

Jackson IMD, Reichlin S. Thyrotropin releasing hormone (TRH) distribution in the brain, blood and urine of the rat. Life Sci 1974a;14:2259–2266. [PubMed: 4211252]

Jackson IMD, Reichlin S. Distribution of pGlu-His-ProNH_2 ("TRH") in hypothalamic and extrahypothalamic tissues of mammalian and submammalian Chordates. Endocrinology 1974b; 95:816–824.

Kalent H, Grose W. Effects of ethanol and pentobarbital on release of acetylcholine from cerebral cortex slices. J. Pharmacol. Exp. Ther 1967;158:386–393. [PubMed: 5624079]

Keller HH, Bartholini G, Pletscher A. Enhancement of cerebral noradrenaline turnover by thyrotropin-releasing hormone. Nature (London) 1974;248:528–529. [PubMed: 4207412]

Koelle, GB. Neuromuscular blocking agents. In: Goodman, LS.; Gilman, A., editors. The Pharmacological Basis of Therapeutics. The Macmillan Company; New York: 1970. p. 601-619.

May P, Donabedian RK. Factors in blood influencing the determination of thyrotropin-releasing hormone. Clin. Chim. Acta 1973;46:377–382. [PubMed: 4201253]

Mujic M, Van Rossum JM. Comparative pharmacodynamics of sympathomimetic imidazolines: Studies on intestinal smooth muscle of the rabbit and the cardiovascular system of the cat. Arch. Int. Pharmacodyn. Ther 1965;155:432–449. [PubMed: 5841192]

Nair RMG, Redding TW, Schally AV. Site of inactivation of thyrotropin-releasing hormone by human plasma. Biochemistry 1971;10:3621–3624. [PubMed: 5004409]

Phillis JW, Jhamandas K. The effects of chlorpromazine and ethanol on *in vivo* release of acetyicholine from the cerebral cortex. Comp. Gen. Pharmacol 1971;2:306–310. [PubMed: 5162666]

Piva, F.; Steiner, H. Bioassay and Toxicology of TRH. In: Hall, R.; Werner, I.; Holgate, H., editors. Frontiers of Hormone Research: Thyrotropin Releasing Hormone. S. Karger; Basel: 1971. p. 11-21.

Plotnikoff NP, Prange AJ Jr. Breese GR, Anderson MS, Wilson IC. Thyrotropin-releasing hormone: Enhancement of DOPA activity by a hypothalamic hormone. Science (Washington) 1972;178:417–418. [PubMed: 4627781]

Prange AJ, Breese GR, Cott JM, Martin BR, Cooper BR, Wilson IC, Plotnikoff NP. Thyrotropin releasing hormone: Antagonism of pentobarbital in rodents. Life Sci 1974;14:447–455. [PubMed: 4207373]

Prange AJ Jr. Breese GR, Jahnke GD, Cooper BR, Cott JM, Wilson IC, Liption MA, Plotnikoff NP. Parameters of alteration of pentobarbital response by hypothalamic polypeptides. Neuropsychobiology 1975;1:121–131. [PubMed: 817220]

Reigle TG, Avni J, Platz PA, Schildkraut JJ, Plotnikoff NP. Norepinephrine metabolism in the rat brain following acute and chronic administration of thyrotropin releasing hormone. Psychopharmacologia 1974;37:1–6. [PubMed: 4213267]

Sunshine I, Nenad R. A modification of Winnick's method for the rapid determination of ethyl alcohol in biological fluids. Anal. Chem 1953;25:653–655.

Tata, JR. Biological action of thyroid hormones at the cellular and molecular levels. In: Litwack, G.; Kritchevsky, D., editors. Actions of Hormones on Molecular Processes. John Wiley & Sons, Inc.; New York: 1964. p. 58-131.

Taurog A, Oliver C, Eskay RL, Porter JC, McKenzie JM. The role of TRH in the neoteny of the Mexican axolotl. Gen. Comp. Endocrinol 1974;24:267–279. [PubMed: 4214731]

Vale W, Burgus R, Dunn TF, Guillemin R. Plasma inactivation of thyrotropin releasing factor (TRF) and related peptides: Its inhibition by various means and by the synthetic dipeptide PCAHIS-OME. Hormones (Basel) 1971;2:193–203.

Vale, W.; Grant, G.; Guillemin, R. Chemistry of the hypothalamic releasing factors–Studies on structure-function relationships. In: Ganong, WF.; Martini, L., editors. Frontiers in Neuroendocrinology. Oxford University Press; New York: 1973. p. 375-413.

Volicer L, Gold BI. Effect of ethanol on cyclic AMP levels in the rat brain. Life Sci 1973;13:269–280. [PubMed: 4356352]

Volle, RL.; Koelle, GB. Ganglionic stimulating and blocking agents. In: Goodman, LS.; Gilman, A., editors. The Pharmacological Basis of Therapeutics. The Macmillan Company; New York: 1970. p. 585-600.

Winokur A, Utiger RD. Regional distribution of thyrotropin releasing hormone in rat brain. Science (Washington) 1974;185:265–267. [PubMed: 4209271]

TABLE 1
Effect of TRH on ethanol-induced sleep in mice

TRH was administered intracisternally 10 minutes after injection of 4.7 g/kg of ethanol. TRH administered intraperitoneally or orally was given at the time of ethanol administration. Linear correlation coefficients for sleep time over log dose of TRH: Intracisternal: $r = 0.976$, $n = 8$, $P < .001$. Intraperitoneal: $r = 0.844$, $n = 5$, $P > .05$. Oral: $r = 0.686$, $n = 5$, $P > .1$

Treatment	*N*	Sleeping time
		min ± S.E.M.
A. Intracisternal TRH		
Saline	24	93.7 ± 5.93
0.01 μg	9	72.5 ± 11.57
0.05 μg	10	67.4 ± 7.41
0.2 μg	17	65.2 ± 7.99[a]
1.0 μg	17	62.7 ± 7.96[b]
5.0 μg	15	55.6 ± 5.78[e]
10.0 μg	18	51.6 ± 4.74[e]
15.0 μg	15	48.3 ± 5.90[e]
20.0 μg	14	51.9 ± 4.99[e]
B. Intraperitoneal TRH		
Saline	19	79.5 ± 6.58
1 mg/kg	9	68.8 ± 12.14
3 mg/kg	10	58.0 ± 7.20
10 mg/kg	10	49.5 ± 5.30[c]
30 mg/kg	10	56.6 ± 5.02[a]
100 mg/kg	10	46.2 ± 2.98[e]
C. Oral TRH		
Saline	31	68.6 ± 5.66
1 mg/kg	10	58.8 ± 7.33
3 mg/kg	10	75.3 ± 4.33
10 mg/kg	27	50.3 ± 4.01[a]
30 mg/kg	31	49.4 ± 2.83[c]
100 mg/kg	30	46.4 ± 3.18[d]

When compared with saline:

[a] $P < .05$.

[b] $P < .02$.

[c] $P < .01$.

[d] $P < .005$.

[e] $P < .001$.

TABLE 2

Effects of TRH on ethanol-induced sleep and hypothermia in various species

All animals received TRH (10 mg/kg i.p.) simultaneous with ethanol. Rectal temperatures were determined in rats and hamsters 40 minutes, in guinea pigs 60 minutes, and in gerbils 30 minutes after TRH injection

Animal	Ethanol Dose	*N*	Sleeping Time		Rectal Temperature	
			Saline	TRH	Saline	TRH
g/kg			*min ± S.E.M.*		*° C ± S.E.M.*	
Rat	3.5	16	89.3 ± 5.81	59.2 ± 8.74[a]	37.1 ± 0.13	36.9 ± 0.24
Guinea pig	3	7	139.3 ± 6.94	14.0 ± 1.22[b]	36.4 ± 0.30	36.9 ± 0.29
Hamster	4	9	158.6 ± 8.69	108.7 ± 5.95[b]	36.1 ± 0.18	37.5 ± 0.09[b]
Gerbil	3.8	18	224.9 ± 25.0	78.4 ± 8.39[b]	36.5 ± 0.39	38.5 ± 0.24[b]

When compared with saline:

[a] $P < .02$.

[b] $P < .01$.

TABLE 3
Role of the thyroid axis in TRH antagonism of ethanol

Hypophysectomized mice received 4.3 g/kg, intact mice 4.7 g/kg of ethanol. All drugs were administered intraperitoneally. Hypophysectomized mice used in this study had pituitaries removed at Charles River Breeding Laboratories 3 days prior to the test. The effectiveness of the procedure was confirmed by holding mice for an additional month to establish that further weight gain did not occur. At that time hypophysectomized mice weighed 20 g and unoperated controls of equal weight at the time of surgery weighed 35 g. Temperature was recorded 40 minutes after ethanol treatment.

Treatment	*N*	Sleeping Time	Rectal Temperature
		min ± *S.E.M.*	*°C* ± *S.E.M.*
Hypophysectomized mice			
Saline	9	58.8 ± 9.60	32.9 ± 0.39
TRH, 10 mg/kg	10	31.2 ± 2.75[b]	34.0 ± 0.27[a]
Intact mice			
Saline	9	75.9 ± 5.36	36.3 ± 0.09
Triiodothyronine 1 mg/kg	10	75.9 ± 13.15	36.5 ± 0.19
Thyrotropin, 10 mg/kg	10	79.2 ± 8.85	36.5 ± 0.19
TRH, 10 mg/kg	10	44.0 ± 394[c]	37.0 ± 0.10[c]

When compared with saline:

[a] $P < .05$.

[b] $P < .02$.

[c] $P < .001$.

TABLE 4

Effects of intracisternally administered TRH analogs and other hypothalamic peptides on ethanol-induced sleep and hypothermia in mice

Drug dose was 24 nmol in 10 μl of saline; doses were injected intracisternally 10 minutes after ethanol (4.7 g/kg). Rectal temperature was recorded 40 minutes after ethanol treatment.

Treatment[a]	*N*	Sleeping Time	Rectal Temperature
		min ± *S.E.M.*	*°C* ± *S.E.M.*
Saline	59	99.5 ± 5.1	35.1 ± 0.13
TRH	30	61.2 ± 3.6[c]	37.1 ± 0.16[c]
Pyrazolyl TRH	9	45.0 ± 3.6[c]	37.0 ± 0.21[c]
Lysine TRH	8	61.4 ± 6.1[c]	36.1 ± 0.14[c]
Diiodo TRH	19	65.5 ± 5.4[c]	36.0 ± 0.15[c]
β-AlanineTRH	9	61.8 ± 6.7[c]	37.0 ± 0.19[c]
Deamidated TRH	10	99.3 ± 12.9	36.1 ± 0.11[c]
MIF	8	111.6 ± 7.9	35.4 ± 0.13
SRIF	16	90.9 ± 10.3	36.4 ± 0.19[c]
LHRH	20	93.1 ± 8.0	35.5 ± 0.17
Substance P	31	69.1 ± 6.9[b]	35.7 ± 0.20
OMeDP	20	105.7 ± 7.5	35.0 ± 0.31
OMeDP + TRH	18	48.1 ± 3.6[d]	36.6 ± 0.19[c]

[a] TRH = pyroGlu-His-Pro-NH_2; pyrazolyl TRH = pyroGlu-(pyrazolyl-3) Ala-Pro-NH_2; lysine TRH = pyroGlu-Lys-Pro-NH_2; β-alanine TRH = pyroGlu-His-Pro-βAla-NH_2; diiodo TRH = pyroGlu-2,4-diiodo-His-Pro-NH_2; deamidated TRH = pyroGlu-His-Pro; MW = Pro-Leu-Gly-NH_2; SRIF = Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys-OH; LHRH = pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2; substance P = Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2; OMeDP = pyro-Glu-His-OME.

[b] When compared with saline, $P < .01$.

[c] When compared with saline, $P < .001$.

[d] When compared with TRH alone, $P < .02$.

TABLE 5
Effect of TRH on brain content of ethanol

TRH was administered intraperitoneally immediately after ethanol. P > .05 at all time periods when TRH treated group is compared with the group that received only ethanol.

Treatment	*N*	Ethanol		
		30 min	60 min	90 min
		mg/g of brain ± S.E.M.		
Ethanol (4.7 g/kg)	10	4.89 ± 0.11	4.51 ± 0.10	4.33 ± 0.07
Ethanol + TRH (10 mg/kg)	10	4.69 ± 0.11	4.40 ± 0.09	4.07 ± 0.11

TABLE 6

Effects of noradrenergic agonists on ethanol-induced sleep and noradrenergic antagonists on TRH reduction of ahanol-induced sleep in mice

All mice received 4.6 g/kg of ethanol 10 minutes before intracisternal drug treatments. Each group contained 8 to 60 mice. α-Methyltyrosine, ethanol and TRH were administered intraperitoneally; all other drugs were injected intracisternally and are reported as total dose per brain. Rectal temperature was measured 40 minutes after ethanol administration. When pretreatment groups (Saline, α-methyltyrosine, propranolol, phentolamine) are compared to corresponding group plus TRH, a percent sleep reduction by TRH is obtained. These values are: saline + TRH = 29.5 + 4.2%; a-methyltyrosine + TRH = 50.7 ± 6.6% ($P < .05$); 35 μg of propranolol + TRH = 31.4 ± 6.6%; phentolamine + TRH = 58.4 + 5.2% ($P < .001$). When similar comparisons are made for percent antagonism of hypothermia, the values are: saline + TRH = 4.3 ± 0.47%; α-methyltyrosine + TRH = 3.9 ± 0.59%; 35 μg of propranolol + TRH = 2.2 ± 0.38% ($P < .005$); phentolamine + TRH = 1.0 ± 0.33% ($P < .001$)

Treatment	Dose	Sleeping Time	Rectal Temperature
		min ± *S.E.M.*	*°C* ± *S.E.M.*
A. Noradrenergic agonists			
Saline		91.0 ± 4.8	35.6 ± 0.14
L-Norepinephrine	0.2 μg	103.6 ± 7.1	35.6 ± 0.26
	5 μg	113.9 ± 14.3	37.3 ± 0.17[d]
	30 μg	150.0 ± 15.0[c]	35.5 ± 0.27
Xylometazoline	0.2 μg	138.9 ± 17.9	34.5 ± 0.29[a]
	5 μg	109.4 ± 17.3	35.0 ± 0.19
	20 μg	132.6 ± 13.5	34.8 ± 0.17[b]
d-Amphetamine	4 μg	91.4 ± 9.5	35.1 ± 0.33
	8 μg	80.6 ± 10.8	36.8 ± 0.45
	50 μg	76.6 ± 7.9	37.3 ± 0.32[d]
	100 μg	93.2 ± 8.9	38.2 ± 0.24[d]
B. Noradrenergic antagonists and TRH			
Saline		82.8 ± 4.4	35.6 ± 0.10
TRH	10 mg/kg	51.8 ± 35[c]	37.1 ± 0.17[d]
α-Methyltyrosine	300 mg/kg	83.0 ± 9.5	35.5 ± 0.21
α-Methyltyrosine + TRH	300 mg/kg + 10 mg/kg	40.9 ± 55[e]	37.0 ± 0.21[g]
dl-Propranolol	5 μg	98.5 + 12.3	
	10 μg	63.3 ± 5.3[d]	35.1 ± 0.21
	20 μg	77.8 ± 5.1	34.6 ± 0.20[d]
	35 μg	83.3 ± 7.2	35.1 ± 0.19
dl-Propranolol + TRH	10 μg + 10 mg/kg	40.6 ± 3.8[g]	36.4 ± 0.19[g]
	20 μg + 10 mg/kg	55.6 ± 5.8[f]	35.7 ± 0.28[g]
	35 μg + 10 mg/kg	60.3 ± 5.6[e]	35.8 ± 0.12[g]
Phentolamine	2 μg	147.4 ± 19.3[d]	35.5 ± 0.11
Phentolamine + TRH	2 μg + 10 mg/kg	61.7 ± 77[g]	36.0 ± 0.12[f]

When compared with saline:

When compared with treated group without TRH:

[a] P < .05.

[b] P < .025.

[c] P < .005.

[d] P < .001.

[e] P < .02.

[f] P < .01.

[g] p < .005.

TABLE 7

Effects of intracisternally administered atropine methyl nitrate on reduction of ethanol-induced sleep by TRH

Treatment	Dose	Sleeping Time	
		Saline Pretreat-ment[a]	Atropine Methyl Nitrate Pretreat-ment[a]
		min ± *S.E.M.*	
Saline		109.4 ± 8.0 (19)	110.4 ± 6.9 (28)
TRH	10 mg/kg	69.3 ± 4.3[d] (20)	117.8 ± 5.4 (19)
TRH	30 mg/kg	73.1 ± 3.2[d] (24)	90.7 ± 5.0 (10)
TRH	100 mg/kg	80.1 ± 5.1[b] (24)	97.8 ± 3.2 (10)
TRH	5 μg (i.c.)	76.5 ± 5.1[c] (10)	101.3 ± 11.1 (9)

When compared with i.p. saline treatment:

[a] Atropine methyl nitrate (10 μg) and saline pretreatments were administered intracisternally (i.c.) 10 minutes after ethanol, and 30 seconds before TRH. Number of animals per group appear in parentheses.

[b] $P < .025$.

[c] $P < .01$.

[d] $P < .001$.

TABLE 8

Effects of drugs which affect cholinergic mechanisms on ethanol-induced sleep and sleep reduction produced by TRH

All mice received 4.7 g/kg of ethanol. TRH (10 mg/kg) was administered intraperitoneally 10 minutes after ethanol. All other compounds including atropine methyl nitrate (AMN) were administered intracisternally (i.e.) 10 minutes after ethanol.

Treatment	Dose	*N*	Sleeping Time
			min ± S.E.M.
Saline		47	104.7 ± 5.0
TRH	10 mg/kg	36	56.6 ± 4.0[b]
Atropine methyl nitrate (AMN)	10 μg	19	95.8 ± 6.2
d-Tubocurarine	5 μg	19	32.5 ± 3.1[b]
d-Tubocurarine + TRH	5 μg + 10 mg/kg	9	20.8 ± 1.9[b,e]
d-Tubocurarine	3 μ	9	51.9 ± 4.7[b]
d-Tubocurarine + AMN	3 μg + 10 μg	9	44.2 ± 2.6[b]
Gallamine	5 μg	18	43.7 ± 3.3[b]
Gallamine + TRH	5 μg + 10mg/kg	9	28.1 ± 1[b,e]
Gallamine	3 μg	10	56.4 ± 7.0[b]
Gallamine + AMN	3 μg + 10 μg	10	70.2 ± 8.6[a]
Hexamethonium	l00 μg	20	76.7 ± 7.2[a]
Hexamethonium + TRH	100 μg + 10 mg/kg	18	49.9 ± 6.4[a,d]
Hexamethonium + AMN	l00 μg + 10 μg	10	95.6 + 14.04
Carbachol	0.3 μg	28	74.9 ± 4.9[b]
Carbachol + TRH	0.3 μg + 10mg/kg	20	103.1 ± 11.7
Carbachol + AMN	0.3 μg + l0 μg	20	93.2 ± 5.5[c]

When compared with saline:

When compared with appropriate group that received only *d*-tubocurarine, gallamine, hexamethonium or carbachol:

[a] P < .02.

[b] P < .001.

[c] P < 0.5

[d] P < .01.

[e] P < .001.

TABLE 9

Effects of theophylline and dibutyryl cyclic AMP on the antagonism of ethanol by TRH

All mice received 4.7 g/kg of ethanol intraperitoneally. In Study A, theophylline and TRH were administered intraperitoneally. In Study B, dibutyryl cyclic AMP and atropine methyl nitrate (AMN) were administered intracisternally while TRH was administered intraperitoneally. Each group contains from 8 to 49 animals. Rectal temperature was determined 40 minutes after ethanol injection.

Treatment	Sleeping Time	Rectal Temperature
	min ± *S.E.M.*	*°C* ± *S.E.M.*
Study A.		
Control	71.2 ± 5.8	35.9 ± 0.19
Theophylline (10 mg/kg)	60.1 ± 7.5	36.0 ± 0.17
Theophylline + TRH	32.7 ± 3.5[c]	36.9 ± 0.17[d]
TRH (10 mg/kg)	43.4 ± 4.5[c]	36.7 ± 0.16[d]
Study B.		
Control	80.9 ± 2.4	36.2 ± 0.10
TRH (10 mg/kg)	56.1 ± 4.1[d]	37.0 ± 0.11[d]
Dibutyryl cAMP (20 μg)	57.0 ± 2.6[d]	36.1 ± 0.21
Dibutyryl cAMP + TRH	51.2 ± 7.3[c]	37.4 ± 0.21[d]
Dibutyryl cAMP + AMN (10 μg)	68.8 ± 3.7[d]	35.8 ± 0.22
Dibutyryl cAMP + AMN (20 μg)	61.3 ± 6.8[a,f]	34.8 ± 0.28[d,f]
AMN (10 μg)	79.3 ± 4.6	36.4 ± 0.25
AMN(20 μg)	95.8 ± 5.8[b]	36.2 ± 0.12

When compared with control:

When compared with AMN alone:

[a] P < .05.

[b] P < .025.

[c] P < .005.

[d] P < .001.

[e] P < .05.

[f] P < .001.