The Neuropeptides
Pharmacology Biochemistry and Behavior, Vol. 5, Suppl. 1, pp. 129–133. ANKHO International Inc., 1976.

Regulation of Behavioral Events by Thyrotropin Releasing Factor and Cyclic AMP

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COHN, M. L., M. COHN, B. A. KRZYSIK AND F. H. TAYLOR. *Regulation of behavioral events by thyrotropin releasing factor and cyclic AMP.* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 129–133, 1976. Like dibutyryl cyclic AMP, thyrotropin releasing factor (TRF) has potent antianesthetic properties, but only dibutyryl cyclic AMP shortens narcosis dose-relatedly. In contrast, only TRF reverses amobarbital-induced hypothermia (dose-relatedly). In naive rats, dibutyryl cyclic AMP (25–200 μg) induces convulsions while TRF (5–100 μg) produces intermittent hyperactivity and sedation but never convulsions. To determine whether behavioral events may be regulated in the central nervous system through an interaction of the two naturally occurring compounds, TRF (5–100 μg) and dibutyryl cyclic AMP (25–200 μg) were injected simultaneously into the lateral ventricle of the brain of naive rats or rats anesthetized with amobarbital (80 mg/kg). TRF (12.5–50 μg) and dibutyryl cyclic AMP (100–200 μg) did not shorten narcosis further than dibutyryl cyclic AMP alone. Amobarbital protected against the lethal effects of the two compounds injected simultaneously. Long-lasting locomotor disorders and mortality rate increased with increasing doses of TRF (12.5–25 μg) and dibutyryl cyclic AMP (100–200 μg) given to naive rats. Results did not support the postulate that cyclic AMP is the second messenger of TRF.

Cyclic AMP Thyrotropin releasing factor Locomotor regulation Temperature regulation

THERE is increasing evidence that besides inducing anterior pituitary secretions, TRH, the thyrotropin releasing hormone, also regulates extrapituitary functions of the brain. (For our present study, we prefer the term TRF-thyrotropin releasing factor, because we wish to remove the suggestion that the behavioral events we report are mediated through the pituitary-thyroid axis). While radioimmunoassays show that TRF is located throughout the brain [20, 27, 37, 38] and the cerebrospinal fluid [22], its 14 to 103 fold higher concentrations in the hypothalamus suggest a primarily endocrine function in the central nervous system. Our amino acid analyzer technique shows that the higher concentrations of TRF in the hypothalamus are duplicated in the caudate nucleus and cerebral cortex [23]. The contention that TRF is a neurotransmitter or modifier of neural conduction is supported by recent workers who 1) detected TRF at synaptic junction sites where norepinephrine, dopamine and acetylcholine are found [1,3]; 2) demonstrated that TRF directly depresses neuronal conduction [34,35]; and 3) showed that TRF has direct pharmacologic behavioral effects in the central nervous system [6, 10, 31]. It is also known that TRF stimulates adenyl cyclase in the adenohypophysis [24,40]. In order to gain a better understanding of the relationship of TRF and the adenyl cyclase system, we undertook the present study.

METHOD

Male Sprague-Dawley rats (Zivic Miller, Pittsburgh, Pennsylvania) weighing 85–125 g were housed under constant illumination and temperature (21°C). Prior to the experiment, they were allowed free access to water and rat chow. All treatments were carried out between 9 a.m. and noon.

Rats were anesthetized with a 1% solution of amobarbital (Amytal) (E. Lilly Company, Indianapolis, Indiana) (80 mg/kg) injected intraperitoneally (IP). The time from the loss of the righting reflex until the recovery of the righting reflex was measured in minutes and defined as sleeping time. Descriptive statistics for the sleeping times for various treatment groups are given in Table 1. Following the loss of the righting reflex, a constant volume of 15 μl was injected intracerebroventricularly (ICV) by a method described elsewhere [13]. The compounds administered centrally, either alone or simultaneously, include: the N^6, O^2, dibutyryl analog of adenosine 3′,5′ cyclic monophosphate (dibutyryl cyclic AMP) (Sigma Chemical Co., St. Louis, Missouri); thyrotropin releasing factor (TRF) (generously provided by Abbott Laboratories, Chicago, Illinois and checked for purity by amino acid analysis and thin layer chromatography); or sterile sodium chloride 0.9%. Dibutyryl cyclic AMP and TRF were diluted in 10^{-3}M phosphate buffer pH 7.3. Rectal temperatures were recorded at 5 min intervals with a thermister probe (Baily Instruments Company, Saddle Brook, New Jersey). All behavioral activities of the rats were carefully observed and recorded on the days of the experiments and for 5 days thereafter.

The rats were divided into 4 treatment groups and subdivided into subgroups of 10 rats for each dosage studied:

1. Control groups: naive rats were injected ICV with sodium chloride 0.9% or various doses of TRF (5–100 μg/rat).

2. Anesthetized rats received ICV sodium chloride 0.9% or TRF (12.5–100 μg/rat).

3. Naive rats were injected ICV simultaneously with TRF and dibutyryl cyclic AMP: a) TRF (5.0 μg/rat) and dibutyryl cyclic AMP (25 μg/rat); b) TRF (12.5 μg/rat) and dibutyryl cyclic AMP (100 μg/rat); c) TRF (25–100 μg/rat) and dibutyryl cyclic AMP (100–200 μg/rat).

4. Anesthetized rats were administered ICV with one of various solutions of TRF (12.5–50 μg/rat) and dibutyryl cyclic AMP (100–200 μg/rat).

RESULTS

In naive rats, sodium chloride produced no noticeable behavioral changes. Within 1 min of injection, the various doses of TRF (5–100 μg/rat) produced intermittent hyperactivity lasting less than 10 min and deep sedation of about 30 min duration. Hyperactivity was characterized by increased locomotor activity and body tone, tail lifting, squirrel upright position, frequent grooming, fasciculations and spasms of muscles in the extremities, scratching, hyperventilation, piloerection, blinking and tearing. Locomotor movements seemed exaggerated, with arched backs and staggering gait. Intensity and duration of the symptoms were not dose-related even at higher doses (100–200 μg/rat). Neither convulsions nor mortality were observed in these groups of rats. While sodium chloride produced no alterations of body temperature, TRF elevated the body temperature above control values for well over 2 hr (Fig. 1).

Anesthetized rats treated with sodium chloride injected centrally regained the righting reflex in the control time of 111.5 min (Table 1). The various doses of TRF (12.5–100 μg/rat) shortened sleeping time, but not dose-relatedly (Table 1). The wide range of sleeping times and behavioral responses to TRF were characteristic. Rats anesthetized with amobarbital are flaccid and still until regaining the righting reflex; within 2 min of injection, TRF induced shivering, shaking of the head and body as though wet, scratching, spontaneous tail movements, piloerection, hyperventilation, blinking, tearing and a rapid return of body tone. Upon return of the righting reflex, the rats exhibited about 30 min of mild ataxia characterized by staggering gait and arched back. Sedation slowly improved and one hour later the rats resumed normal behavior. The body temperature of amobarbital-anesthetized rats normally fell 2.87°C in 60 min (Table 1). Sodium chloride had no effect on body temperature. At all doses (12.5–100 μg/rat), TRF antagonized the amobarbital-induced hypothermia and rapidly returned it to normal values; higher doses elevated body temperature above control values (Table 1). No deaths were recorded in these groups of rats even at doses of TRF as large as 200 μg/rat. The prior injection of TRF did not delay the onset of narcosis.

In naive rats, the simultaneous injections of TRF (5.0 μg/rat) and dibutyryl cyclic AMP (25 μg/rat) produced a behavioral pattern similar to that induced by TRF alone. However, all symptoms were enhanced and of longer duration (1–2 hr). No rats of this treatment group died. Besides frequent grooming, marked hyperventilation, blinking and tearing, TRF (12.5 μg/rat) and dibutyryl cyclic AMP (100 μg/rat) produced 10 min of severe clonic-tonic convulsions during which one third of the rats died. The survivors of these convulsions experienced an intense and long-lasting syndrome of locomotor incoordination and hyperactivity marked by severe spasms and fasciculations of the limb muscles and a toe spreading that caused an ataxic gait exaggerated to the point of belly crawling, with rolling, twisting movements of the torso and slapping of the tail from side to side. The locomotor incoordination lasted well over one hour, diminishing gradually with time. After several hours of deep sedation, the rats resumed normal behavior and did not show any sequellae thereafter. Within minutes, TRF (25 μg/rat) and dibutyryl cyclic AMP (100–200 μg/rat) produced intermittent short periods of deep sedation and convulsions characterized by high jumps, phonation, Straub tail phenomenon with high speed vibrations of tail and whiskers, exophthalmia, salivation, piloerection and tearing. Within 30 to 60 min, all the rats of this group died with instant marked rigor mortis.

In the naive rats, dibutyryl cyclic AMP (25 μg/rat) did

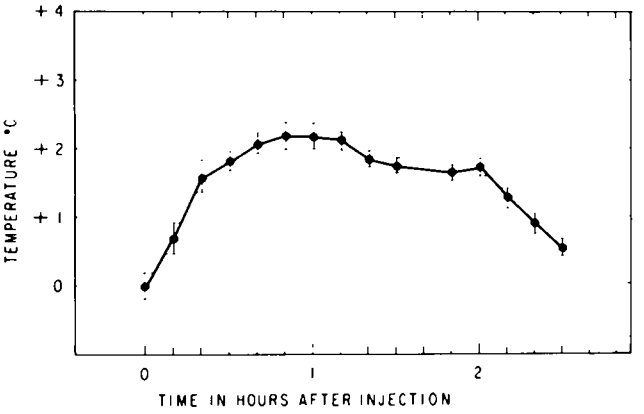

FIG. 1. Mean change in rectal temperature following central injection of thyrotropin releasing factor (50 μg). Room temperature 21.5°C. Treatment group contains 6 rats.

TABLE 1

EFFECT OF TRH (15 μl) ADMINISTERED INTRACEREBROVENTRICULARLY ON THE DURATION OF AMOBARBITAL-INDUCED NARCOSIS (80 mg/kg)

Treatment	N	Sleeping Time min ± S.E.M.	Rectal Temperature * °C ± S.E.M.
(Control – Saline [0.9%])	10	111.5 ± 12.5	2.87 ± 0.21
TRF (12.5 μg)	10	69.5 ± 13.8 ‡	1.25 ± 0.18
TRF (25 μg)	10	69.9 ± 15.0 ‡	+0.20 ± 0.06
TRF (50 μg)	10	62.4 ± 12.0 ‡	+0.87 ± 0.25
TRF (100 μg)	10	65.7 ± 15.8 ‡	+1.88 ± 0.19

*Rectal temperature (°C) measured 30 min after amobarbital administration.

‡Based on the Mann-Whitney U test, statistically significant difference at 0.05 level of significance when compared with control group.

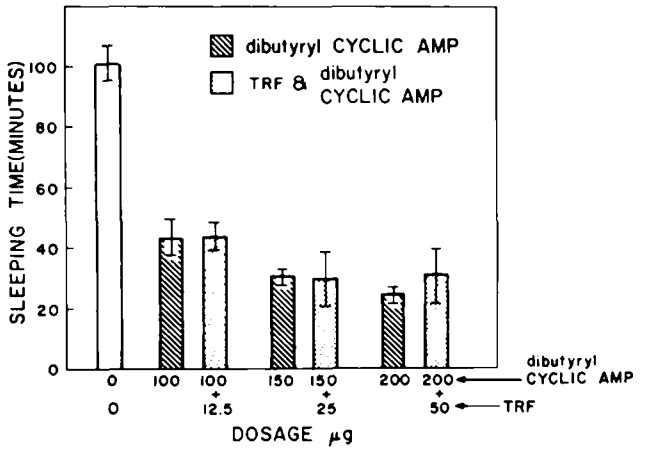

FIG. 2. Comparison of sleeping times in rats anesthetized with IP administered amobarbital (80 mg/kg) and subsequently treated ICV with dibutyryl cyclic AMP alone or simultaneous injections of dibutyryl cyclic AMP and TRF. At the 3 concentrations of dibutyryl cyclic AMP and dibutyryl cyclic AMP combined with TRF, all paired comparisons of the mean sleeping times are statistically significant in comparison to the control sleeping time at the 0.05 level of significance using the Student-Newman-Kreuls test. At the 0.05 level of significance the mean sleeping time of the dibutyryl cyclic AMP treated group is not significantly different when compared with the mean sleeping times of the groups treated simultaneously with dibutyryl cyclic AMP and TRF. Each group contains 10 rats.

not alter the elevation of body temperature induced by TRF alone. Severe convulsions produced by higher dosages of TRF in solution with dibutyryl cyclic AMP did not allow the recording of body temperature.

The simultaneous administration of various doses of TRF (12.5–50 μg/rat) and dibutyryl cyclic AMP (100 200 μg/rat) shortened amobarbital-induced narcosis (80 mg/kg) but not further than dibutyryl cyclic AMP alone (Fig. 2) and produced the same behavioral symptoms as those induced by TRF alone, but these symptoms were much more pronounced. Upon the return of the righting reflex, the rats experienced an acute locomotor cerebral syndrome characterized by twisting gyrations of the body, severe ataxia of the hind limbs, loud tail slapping, fasciculations and muscle spasms of the front limbs and paws, spreading of the toes, random, aimless wandering over the entire bench top, bumping into objects or falling off the bench that lasted for at least 3 hr. When the initial gyrations ended, the rats belly crawled, and after a slow recovery of usage of the hind legs, walked on tip toes. While pinching the tail revealed that spinal reflexes were intact, the rats were unable to swim when placed in a water tank and sank to the bottom. The period of hyperactivity was followed by long sedation. Amobarbital protected against the lethal combination of the nucleotide and the neurohormonal factor; no convulsions or death occurred in these groups of rats. The following morning the rats behaved normally without any locomotor sequellae. The prior simultaneous injection of TRF and dibutyryl cyclic AMP did not block the onset of narcosis. Like TRF alone, TRF and dibutyryl cyclic AMP antagonized the amobarbital-induced hypothermia.

DISCUSSION

The neurochemical determinants of narcosis and sleep are poorly understood. In the rat, dibutyryl cyclic AMP regulates narcosis induced by amobarbital [13] and 7 other chemically unrelated compounds [11]. The regulatory property of dibutyryl cyclic AMP, "the second messenger", suggested that a neurotransmitter or hormone may serve as a first messenger. The catecholamines, biogenic amines or hormones, shown *in vitro* to elevate brain levels of cyclic AMP, failed to shorten *in vivo* narcosis [6,9]. Likewise, the analeptic drugs, with the exception of picrotoxin, were unsuccessful in shortening narcosis [6]; but all concentrations of picrotoxin produced severe toxicity and mortality.

Workers who reported the antianesthetic properties of TRF suggested that TRF may be the first messenger of cyclic AMP [31]. Our data confirm the antianesthetic property of TRF administered intracerebroventricularly.

However, supporting the report of other investigators [36], we found that the intraperitoneal or intravenous injection of TRF did not shorten pentobarbital-induced (50 mg/kg) narcosis. The failure of peripherally administered TRF to antagonize the barbiturate may be explained by the facts that TRF 1) does not freely cross the cerebrospinal fluid barrier [15, 26, 39]; and 2) has a very short half life in plasma and brain tissue [14,16]. The failure of minimal amounts of exogenously administered TRF to enter the central nervous system may account for the failure to reverse pentobarbital overdosage in rats [5] and for the conflicting results in the TRF treatment of depression in man [2,30]. While we were unsuccessful in treating amobarbital overdosed rhesus monkeys with intravenously injected TRF, centrally injected TRF effectively prevented cardiovascular collapse [12].

Notable differences between dibutyryl cyclic AMP and TRF are evident: 1) unlike dibutyryl cyclic AMP, which shows clear-cut dose relationship at all concentrations, ICV administration of TRF produces widely variable ranges of sleeping times (Table 1); 2) following the ICV administration of dibutyryl cyclic AMP, anesthetized rats remain flaccid and very still until regaining the righting reflex. In contrast, anesthetized rats treated ICV with TRF almost instantaneously shiver, shake heads and bodies as though wet, scratch, move piloerect, hyperventilate, blink and tear; 3) in the naive rat the intracerebroventricular injection of dibutyryl cyclic AMP produces severe generalized convulsions [17]. In contrast, the central administration of TRF leads to intermittent short periods of hyperactivity and sedation but no convulsions [6].

The primary thrust of the present study was to obtain *in vivo* evidence of an interaction between TRF and dibutyryl cyclic AMP in the central nervous system. The data thus gained do not support any simple additive or synergistic interaction. However unclear the specific actions of TRF in the brain, it is clear that TRF is involved in a wide range of behavioral events. For example, TRF increased locomotor activity in pargyline-L-DOPA pretreated rats [28,29]. Similar data were reported in rats pretreated with pargyline-5HT [19]. Such findings suggest that the catecholamines and/or serotonin are involved in the TRF regulation of locomotor behavior. Increased evidence has been reported that the release of TRF is controlled by the catecholamines [32]. Although we have found that dopamine is involved in the TRF regulation of rotational behavior in the rat [10], and other investigators reported that TRF alters dopamine brain content in pargyline-L-DOPA pretreated rats [5], the actions of TRF on the central nervous system are too numerous to be related to

any single neurotransmitter. We have reported that TRF and phentolamine produce tight head to tail rotations [6], and will report that simultaneous administration of dibutyryl cyclic AMP and phentolamine does not induce rotations. Conflicting data have been reported by investigators who found that TRF did not affect the brain content of dopamine, norepinephrine or serotonin or the turnover of norepinephrine [4,33], while other studies showed increased turnover of norepinephrine in rat brain [18,21]. Evidently, more work is needed before the interaction of the adenyl cyclase system, the neurohormonal factor and the catecholamines is fully understood.

Thermoregulation was previously believed to be controlled only by the putative neurotransmitters, e.g. dopamine, norepinephrine or serotonin. Increasing evidence shows that the neurohormones TRF [6, 25, 31] and somatostatin [6,7] regulate body temperature antagonistically. While dibutyryl cyclic AMP does not reverse amobarbital-induced hypothermia, TRF antagonizes the temperature fall and somatostatin produces severe and prolonged hypothermia with temperatures dropping as low as 28°C. However, in rats treated with TRF, the duration of narcosis was not related to body temperature [7].

Although the dissimilarities between the actions of dibutyryl cyclic AMP and TRF do not support a hypothetical first messenger role for TRF in the regulation of narcosis, locomotor activity and temperature, we have demonstrated that TRF is a modulator of many behavioral events; in agreement with other investigators [31], we have reported that T^3, T^4 and TSH do not alter the regulation of narcosis, locomotor activity and temperature or cause unusual behavioral symptoms [6]; that thyroidectomy does not alter the behavioral actions of TRF; confirming our belief that TRF acts in the brain at sites other than the pituitary-thyroid axis.

We have found that somatostatin, luteinizing hormone releasing hormone and substance P also regulate behavioral events in the central nervous system and prolong narcosis [8]. The prevalance of TRF in brain structures and its involvement in behavioral events suggest that the direct effect of TRF on the central nervous system may prove as important as its hormonal role.

ACKNOWLEDGEMENTS

We are indebted to Doctor Myron Taube and Ms. Nadine Darocy for their contributions to this manuscript.

REFERENCES

1. Barnea, A., N. Ben-Jonathan, C. Colston, J. M. Johnston and J. C. Porter. Differential sub-cellular compartmentalization of thyrotropin releasing hormone (TRH) and gonadotropin-releasing hormone (LRH) in hypothalamic tissue. *Proc. natn. Acad. Sci.* **72**: 3153–3157, 1975.
2. Benkert, O., A. Gordon and D. Martschke. The comparison of thyrotropin releasing hormone, luteinizing hormone-releasing hormone and placebo in depressive patients using a double-blind crossover technique. *Psychopharmacologia* **40**: 191–198, 1974.
3. Bennett, G. W., J. A. Edwardson, D. T. Holland, S. L. Jeffcoate and N. White. Release of immunoreactive luteinizing hormone-releasing hormone and thyrotropin-releasing hormone from hypothalamic synaptosomes. *Nature* **257**: 323–325, 1975.
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., 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.
6. Cohn, M. L. Cyclic AMP, thyrotropin releasing factor and somatostatin: Key factors in the regulation of the duration of narcosis. In: *Molecular Mechanisms of Anesthesia*, (Vol. 1), edited by B. R. Fink. New York: Raven Press, 1975, pp. 485–500.
7. Cohn, M. L. and M. Cohn. Thermoregulatory control in the rat anesthetized with amobarbital. Role of thyrotropin releasing factor. American Society of Anesthesiologists, Washington, D. C., October, 1974.
8. Cohn, M. L. and M. Cohn. The neuroendocrine regulation of rotational behavior in the nonlesioned rat by thyrotropin releasing hormone, somatostatin, luteinizing hormone releasing hormone, substance P. *Psychoneuroendocrinology* (in press), 1976.
9. Cohn, M. L., M. Cohn and F. H. Taylor. Norepinephrine an antagonist of dibutyryl cyclic AMP in the regulation of narcosis in the rat. *Res. communs chem. pathol. Pharmac.* **7**: 687–699, 1974.
10. 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.
11. Cohn, M. L., M. Cohn, F. H. Taylor and F. Scattaregia. A direct effect of dibutyryl cyclic AMP on the duration of narcosis induced by sedative, hypnotic, tranquilizer and anaesthetic drugs in the rat. *Neuropharmacology* **14**: 483–487, 1975.
12. Cohn, M. L., S. K. Wolfson, Jr., F. M. Steichen and M. Cohn. Thyrotropin releasing hormone (TRH). An antidote to amobarbital overdosage in the rhesus monkey. *Fedn Proc.* **35**: 564, 1976.
13. Cohn, M. L., H. Yamaoka, F. H. Taylor and B. Kraynack. Action of intracerebroventricular dibutyryl cyclic AMP on amobarbital anaesthesia in rats. *Neuropharmacology* **12**: 401–405, 1973.
14. Dixon, R., S. Fast, M. Buckley and A. Darragh. Disappearance of radioactivity from plasma following intravenous administration of tritiated thyrotropin-releasing hormone (R08-6270) to man. *Experientia* **29**: 483–484, 1973.
15. Dupont, A., F. Labrie, G. Pelletier and R. Puviani. Organ distribution of thyrotropin-releasing hormone. *Gen. comp. Endocr.* **19**: 522–526, 1972.
16. Dupont, A., F. Labrie, L. Levasseur and A. V. Schally. Characteristics of the plasma disappearance of (3H)-thyrotropin-releasing hormone in the rat. *Can. J. Physiol. Pharmac* **52**: 1012–1019, 1974.
17. Gessa, G. L., G. Krishna, J. Forn, A. Tagliamonte and B. B. Brodie. Behavioral and vegetative effects produced by dibutyryl cyclic AMP injected into different areas of the brain. In: *Role of Cyclic AMP in Cell Function, Advances in Biochemical Psychopharmacology*, (Vol. 3), edited by P. Greengard and E. Costa. New York: Raven Press, 1970, pp. 371–381.
18. Horst, W. D. and N. Spirt. A possible mechanism for the antidepressant activity of thyrotropin releasing hormone. *Life Sci.* **15**: 1073–1082, 1974.
19. Huidobro-Toro, J. P., A. Scotti De Carolis and V. G. Longo. Action of two hypothalamic factors (TRH, MIF) and of angiotensin II on the behavioral effects of L-DOPA and 5-hydroxytryptophan in mice. *Pharmac. Biochem. Behav.* **2**: 105–109, 1974.

20. Jackson, I. M. D. and S. Reichlin. Thyrotropin-releasing hormone (TRH): Distribution in hypothalamic and extrahypothalamic brain tissues of mammalian and submammalian chordates. *Endocrinology* **95**: 854–862, 1974.
21. Keller, H. H., G. Bartholini and A. Pletscher. Enhancement of cerebral noradrenaline turnover by thyrotropin-releasing hormone. *Nature* **248**: 528–529, 1974.
22. Knigge, K. M. and S. A. Joseph. Thyrotropin releasing factor (TRF) in cerebrospinal fluid of the third ventricle of rat. *Acta endocr.* **76**: 209–213, 1974.
23. Krzysik, B. A. and M. L. Cohn. Measurement of thyrotropin releasing hormone (TRH) in rat brain by amino acid analysis. *Fedn Proc.* **35**: 778, 1976.
24. Labrie, F., N. Barden, G. Poirier and A. DeLean. Binding of thyrotropin-releasing hormone to plasma membranes of bovine anterior pituitary gland (hormone receptor-adenylate cyclase equilibrium constant-(3H) thyrotropin). *Proc. natn. Acad. Sci. U. S. A.* **69**: 283–287, 1972.
25. Metcalf, G. TRH: A possible mediator of thermoregulation. *Nature* **252**: 310–311, 1974.
26. Morin, A., A. Tixier-Vidal, D. Gourdji, P. Pradelles, J. L. Morgat and P. Fromageot. Cinétique de la répartition d'un TRF tritié à haute radioactivité spécifique (facteur hypothalamique de libération de l'hormone thyréotrope) après injection intraveineuse chez le rat mâle. Comparaison avec les resultats obtenus en incubation. *C. r. Acad. Sci., Paris* **274**: 3283–3286, 1972.
27. Oliver, C., R. L. Eskay, N. Ben-Jonathan and J. C. Porter. Distribution and concentration of TRH in rat brain. *Endocrinology* **95**: 540–546, 1974.
28. Plotnikoff, N. P., A. J. Prange, Jr., G. R. Breese and I. C. Wilson. Thyrotropin releasing hormone: Enhancement of DOPA activity in thyroidectomized rats. *Life Sci.* **14**: 1271–1278, 1974.
29. Plotnikoff, N. P., A. J. Prange, Jr., G. R. Breese, M. S. Anderson and I. C. Wilson. Thyrotropin releasing hormone: Enhancement of DOPA activity by a hypothalamic hormone. *Science* **178**: 417–418, 1972.
30. Prange, A. J., Jr., I. C. Wilson, P. P. Lara, L. B. Alltop and G. R. Breese. Effects of thyrotropin-releasing hormone in depression. *Lancet* **2**: 999–1002, 1972.
31. 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.
32. Reichlin, S. Regulation of hypophysiotropic secretions of the brain. *Arch. intern. Med.* **135**: 1350–1361, 1975.
33. Reigle, T. G., J. Avni, P. A. Platz, J. J. Schildkraut and N. P. Plotnikoff. Norepinephrine metabolism in rat brain following acute and chronic administration of thyrotropin releasing hormone. *Psychopharmacologia* **37**: 1–6, 1974.
34. Renaud, L. P. and J. B. Martin. Thyrotropin releasing hormone (TRH): Depressant action on central neuron activity. *Brain Res.* **86**: 150–154, 1975.
35. Renaud, L. P., J. B. Martin and P. Brazeau. Depressant action of TRH, LH-RH and somatostatin on activity of central neurones. *Nature* **255**: 233–235, 1975.
36. Schenkel-Hulliger, L., W. P. Koella, A. Hartmann and L. Maitre. Tremorgenic effect of thyrotropin releasing hormone in rats. *Experientia* **30**: 1168–1170, 1974.
37. Winokur, A. and R. D. Utiger. Thyrotropin-releasing hormone: Regional distribution in rat brain. *Science* **185**: 265–267, 1974.
38. Winters, A. J., R. L. Eskay and J. C. Porter. Concentration and distribution of TRH and LRH in human fetal brain. *J. clin. Endocr. Metab.* **39**: 960–963, 1974.
39. Witter, A. The in vivo fate of brain oligopeptides. *Biochem. Pharmac.* **24**: 2025–2030, 1975.
40. Zor, U., T. Kaneko, H. P. Schneider and J. Field. Stimulation of anterior pituitary adenyl cyclase activity and adenosine 3′,5′-cyclic phosphate by hypothalamic extract and prostaglandin E.1. *Proc. natn. Acad. Sci. U. S. A.* **63**: 918–925, 1969.