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Intracranial Injection of Thyrotropin Releasing Hormone (TRH) Suppresses Starvation-Induced Feeding and Drinking in Rats

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SUZUKI, T., H. KOHNO, T. SAKURADA, T. TADANO AND K. KISARA. *Intracranial injection of thyrotropin releasing hormone (TRH) suppresses starvation-induced feeding and drinking in rats.* PHARMAC. BIOCHEM. BEHAV. 17(2) 249–253, 1982.—The sites at which TRH produces suppression on feeding and drinking were examined anatomically in the rat brain. This was accomplished by microinjecting nmol concentration of TRH into 6 different brain sites. Intracerebroventricular injection of TRH (25, 50, 100 nmol/rat) suppressed starvation-induced feeding and drinking in a dose related manner. The microinjection in a small amount of TRH (8 nmol/hemisphere) into the medial and lateral hypothalamus produced relatively severe anorexia and adipsia as compared with the other areas including the nucleus accumbens, the substantia nigra, the globus pallidus and the amygdala. It was concluded that the medial hypothalamus is the most sensitive site of TRH-induced anorexia and adipsia and the action of TRH on the lateral hypothalamus is also a possible mechanism mediating the decrease in water intake.

Thyrotropin releasing hormone Anorexia Adipsia Medial hypothalamus Lateral hypothalamus

THYROTROPIN releasing hormone (TRH) is a tripeptide (pyro-glutamyl-histidyl-prolineamide) originally isolated from the hypothalamus [32]. Several recent studies have shown that TRH is present not only in the hypothalamus tissue, but also it is widely distributed throughout the extrahypothalamic nervous system [6, 13, 16, 28, 37, 38]. TRH appears to produce a number of behavioral alterations which are not observed after administration of thyroid-stimulating hormone (TSH) in animals (reviewed in [23,27]). Many of the effects of centrally or peripherally administered TRH appear as increases in arousal. Thus, wide phylogenetic and regional brain distributions of TRH support the hypothesis that, in addition to its role as a hypothalamic hormone, TRH may function as a neurotransmitter. Concerning ingestive behavior, Vijayan and MaCann [34] suggested that TRH may play a physiological role in control of food intake. This view point was supported by studies indicating that TRH given intraperitoneally and intraventricularly produced a significant reduction of food intake [24,35]. And it appears that TRH produces its satiety effect through one of its metabolites, histidyl-proline diketopiperazine [25]. However, the specific brain site of TRH action on feeding and drinking responses is not yet known. Here, we have used intracranial microinjection of TRH into specific brain loci in order to identify the central substrate mediating TRH-induced anorexia and adipsia.

METHOD

Animals

Male Wistar rats, initially weighing 250–300 g, were used. All rats were caged singly in a room kept at 20–24°C, with a 12 hr light, 12 hr dark (lights on 9:00–21:00 hr) environment. Surgery was conducted under sodium pentobarbital (50 mg/kg, IP) anesthesia.

Microinjection

In preparation for intracerebroventricular (ICV) injections, unilateral 23 ga. stainless steel guide cannulas modified hypodermic needle with 27 ga. obdurator were implanted to right lateral ventricle [18]. Bilateral guide cannulas for intracranial injections, stainless steel tubing with an outer and inner diameter of 0.6 and 0.3 mm were implanted stereotaxically into six regions according to the atlas of Pellegrino *et al.* [29], and then guide cannulas were fastened to the skull that the tip of the cannulas was 2 mm above the intended site of injection. ICV injections were made with an injection volume of 10 μl/animal. Intracranial injections were made bilaterally by means of 5 μl micrometer-driver syringe (Hamilton) extending 2 mm below the tips of the guide cannula. A 0.2 μl volume of solution was delivered over 30 sec through each cannula (0.4 μl/animal). After the microinjec-

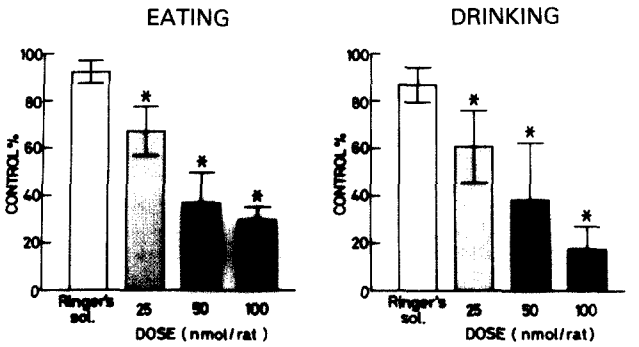

FIG. 1. The effect of intracerebroventricular injection of various amount of TRH in 20 hr food and water deprived rats. The values are mean percentage intake of the amount taken during the 3 previous non-drug days. Each value is the mean±S.E.M. of 5 animals. *Significantly different from Ringer control, $p<0.01$. A ratio of g/kg of food intake to ml/kg of water intake for Ringer control, TRH 25 nmol, 50 nmol and 100 nmol was 1.6±0.2, 2.5±1.3, 1.4±0.4 and 7.1±4.0, respectively.

tion, 10 sec was allowed to elapse before removal of the injection cannula and replacement of the obdurators.

Behavioral Testing

Each rat was placed on a daily 4 hr (16:00–20:00 hr) feeding and drinking schedule following at least a week of the postoperative convalescence. After the rats were well enough accustomed to the schedule feeding and drinking, the administration of TRH dissolved in Ringer's solution or Ringer's solution alone was begun. The powdered food (CE-2 Nippon Clea Co.) and water were made available to rats using glass cup and graduated glass tube, and the amount consumed was measured at 30 min after injection.

Histology

Prior to sacrifice, animals received bilateral microinjection of 0.2% solution of Evans blue dye (5 μl) in order to aid localization of injection sites. They were then overdosed with urethane, intracardially perfused with a 10% formal-saline solution and their brains retained for histological examination. Sections were cut on a freezing microtome and injection sites plotted on line drawings based on the atlas of Pellegrino *et al.* [29].

Statistics

Food and water intake were measured at 30 min, 2 hr, 3 hr and 4 hr after injection. Drug effects were determined by expressing the food intake of each animal on a test day as a percentage of the average intake during the 3 previous non-drug days. The effect of Ringer's solution was never significant. A significant difference between TRH- and Ringer's solution-treated groups was evaluated by Student's t-test at 30 min after injection, since the maximum effect of TRH was observed at 30 min after ICV injection.

RESULTS

ICV Injections of TRH

Control animals administered with 10 μl of Ringer's solution into the ventricle exhibited no significant alteration in their food or water intake compared with non-treated rats. During the first 30 min of the test, ICV injections of TRH produced a marked suppression in starvation-induced eating and drinking in a dose related manner (Fig. 1). TRH at three doses (25, 50 and 100 nmol) was capable of inducing a significant suppression of food or water intake at 30 min. The TRH effect on both ingestive behaviors lasted for 3 hr and disappeared at 4 hr.

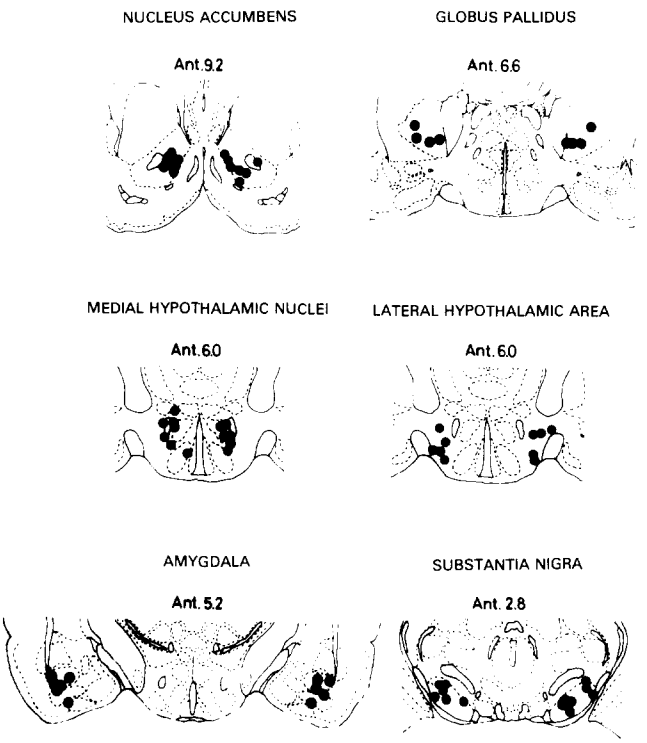

FIG. 2. Schematic summary of injection sites in the medial hypothalamus, lateral hypothalamus, nucleus accumbens, substantia nigra, globus pallidus and amygdala. Line drawings adapted from the atlas of Pellegrino *et al.* [29].

Intracranial Injections of TRH

Histological examination revealed that, in most cases, injection sites were accurately positioned within the desired brain sites. However, in several animals, cannulae were asymmetrically positioned. The data from these animals were discarded from the statistical analyses. Figure 2 illustrates the areas covered by injection within each placement group.

The control rats injected with Ringer's solution intracranially showed a slight alteration of water intake compared with non-treated rats, but a statistically significant difference was not observed. The bilateral application of TRH (8 nmol/hemisphere) into the medial hypothalamic nuclei and accumbens nuclei caused a significant suppression in food consumption (Fig. 3). The medial hypothalamic nuclei was more sensitive to TRH than the accumbens nuclei. TRH into the medial hypothalamic nuclei and accumbens nuclei suppressed food intake for 3 and 2 hours, respectively. However, the other areas including lateral hypothalamic areas

and substantia nigra proved to be insensitive with 8 nmol/hemisphere of TRH. On the other hand, water consumption was significantly reduced by the application of TRH into the medial hypothalamic nuclei, lateral hypothalamic areas and substantia nigra but not into the accumbens nuclei. In the time course observation, the injection of TRH into the medial hypothalamic nuclei, lateral hypothalamic area and substantia nigra produced a suppression of water intake for 2 hr, 1 hr and 30 min, respectively. A significant reduction was not observed following the injection of TRH into the globus pallidus and amygdala on ingestive behavior (Table 1).

DISCUSSION

The present experiments demonstrate that the administration of TRH into the lateral ventricle and brain tissues can produce a decrease in starvation-induced feeding and drinking. These effects produced by administration of TRH into the lateral ventricle, or medial hypothalamic nuclei and accumbens nuclei were not of brief duration, lasting for 3 hours. A relatively long duration of action of TRH on ingestive measures is inconsistent with the brief duration of the analeptic action of TRH against pentobarbital and alcohol narcosis [3, 4, 8, 20]. However, the inhibitory peak time was observed approximately within 30 min. Previous studies have indicated that many actions of TRH are not the result of an activation of the pituitary-thyroid axis [4,30]. Systemically administered TRH increased TRH content in plasma significantly, while even a high dose of thyroid-stimulating hormone administered to food-deprived rats had no significant effects of food ingestion. Therefore, they suggested that the release of TSH was not responsible for the action of TRH to reduce food intake [35].

The present data indicates that ICV injection of TRH suppressed both eating and drinking in a dose related manner. This confirmed the previous studies showing that intraperitoneally, ICV or intra-third ventricularly administered TRH reduced food intake [34,35]. In addition to an action of TRH on food-deprived rats, Morley and Levine have recently reported that TRH injected ICV and parenterally suppressed mild tail-pinch inducing eating [24]. Our results would be in line with the view that this anorectic action of TRH is central in origin.

High concentrations of TRH are found in several hypothalamic nuclei, particularly in the ventromedial nucleus, a region known to be involved in control of food intake and the site of the so-called satiety center [6,37]. And destruction of this area leads to hyperphagia as has been described firstly by Brobeck *et al.* [5] and since then by many others [14]. Though the inhibitory or facilitatory action of iontophoretically applied TRH on various neurons was known [26, 31, 39], Ishibashi *et al.* [15] have recently reported facilitatory effects on glucoreceptive neurons in the ventromedial hypothalamus following the application of TRH. The present results show that the most sensitive brain site was the medial hypothalamus. The evidence supporting their suggestion that the anorectic action of TRH might be produced mainly by facilitation of glucoreceptor neurons in the ventromedial hypothalamus, or several data including immunohistochemical [12,13], chromatographic identification of TRH in the medial hypothalamic region [40] and the demonstration of calcium-dependent release of immunoreactive TRH from medial hypothalamic synaptosomes in the presence of elevated potas-

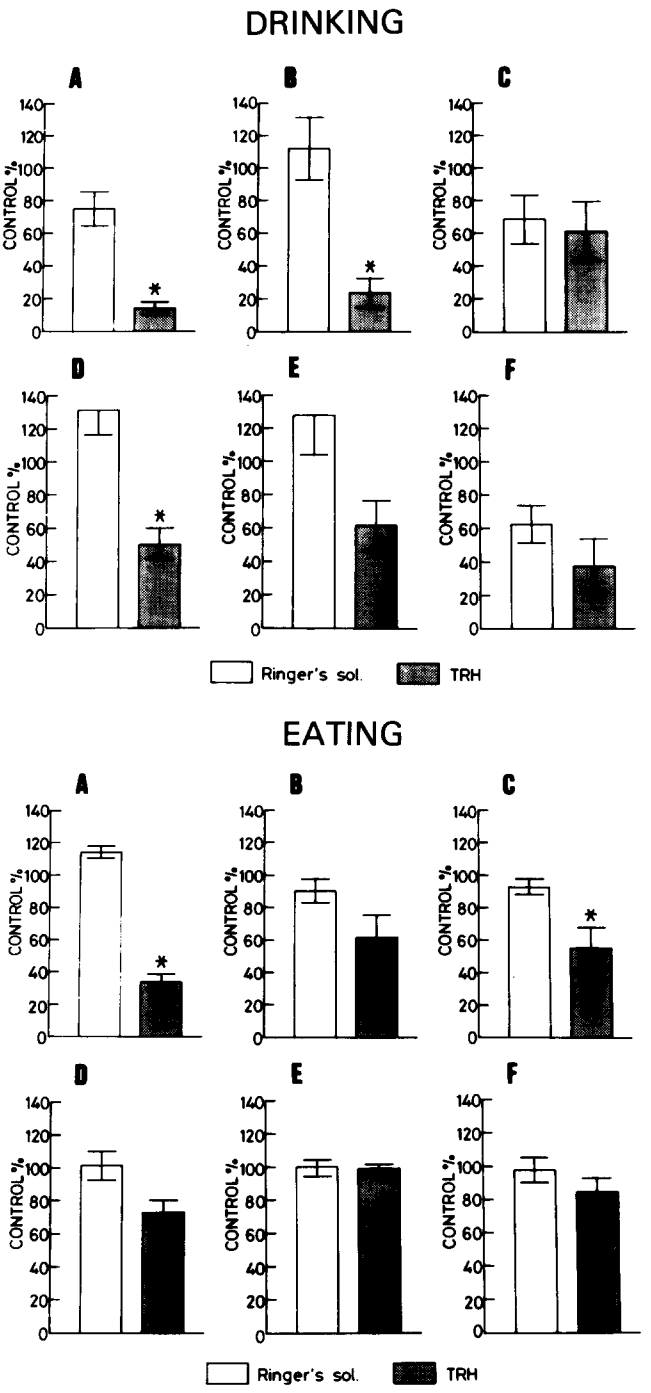

FIG. 3. The effects of bilateral injections of TRH (8 nmol/hemisphere) to the six different regions of the brain in 20 hr food and water deprived rats. The values are mean percentage intake of the amount taken during the 3 previous non-drug days. Each value is the mean±S.E.M. of 4–7 animals. *Significantly different from Ringer solution, $p<0.01$. **Significantly different from Ringer control, $p<0.05$. A: Medial hypothalamus, B: Lateral hypothalamus, C: Nucleus accumbens, D: Substantia nigra, E: Globus pallidus, F: Amygdala. A ratio of g/kg of food intake to ml/kg of water intake for the Ringer control and TRH (8 nmol/hemisphere) was 2.4±0.4, 7.3±2.4 in the medial hypothalamus, 1.0±0.2, 4.4±1.8 in the lateral hypothalamus, 1.3±0.2, 1.2±0.1 in the nucleus accumbens, 1.1±0.1, 2.3±0.5 in the substantia nigra, 1.6±0.2, 2.7±0.6 in the globus pallidus and 1.6±0.3, 6.8±2.7 in the amygdala.

TABLE 1

FOOD AND WATER INTAKE AT 30 MIN, 1 HR, 2 HR, 3 HR and 4 HR AFTER INTRACEREBROVENTRICULAR AND INTRACRANIAL INJECTIONS OF TRH IN 20 HR FOOD AND WATER DEPRIVED RATS

		Food Intake (g)					Water Intake (g)				
		30 min	1 hr	2 hr	3 hr	4 hr	30 min	1 hr	2 hr	3 hr	4 hr
ICV injection											
Ringer	(5)	5.2±0.3	7.9±0.5	10.9±0.7	14.0±0.7	16.6±0.7	3.6±0.4	7.7±0.5	12.5±0.4	16.0±0.5	18.0±0.6
TRH 25 nmol/rat	(5)	3.3±0.5	5.9±0.7	8.5±0.9	11.3±0.4	13.4±1.1	2.5±0.6	6.1±1.0	10.2±1.1	13.1±1.2	16.2±1.7
TRH 50 nmol/rat	(5)	2.2±0.3	3.1±0.5	6.5±0.3	10.9±0.3	13.9±0.8	1.6±1.0	3.5±0.5	9.0±0.6	12.8±1.0	16.5±1.1
TRH 100 nmol/rat	(5)	1.5±0.3	2.7±0.3	5.9±0.5	10.7±0.9	14.4±0.6	0.7±0.4	2.9±0.9	8.0±0.9	11.9±0.8	16.6±1.0
Intracranial injection (8 nmol/hemisphere)											
Medial hypothalamus											
Ringer	(7)	3.4±0.3	6.7±0.3	9.9±0.4	12.3±0.6	13.9±0.9	2.0±0.4	5.2±0.7	9.5±0.5	12.3±0.8	14.7±1.5
TRH	(7)	1.1±0.2	2.6±0.4	5.9±0.8	9.5±1.1	12.2±1.6	0.3±0.1	2.6±0.4	7.2±0.9	12.1±1.4	14.8±1.9
Lateral hypothalamus											
Ringer	(5)	2.3±0.6	4.2±0.8	6.7±0.8	8.3±0.4	11.1±0.7	2.4±0.3	4.9±0.6	8.9±0.5	12.0±0.5	15.1±0.4
TRH	(5)	1.4±0.3	3.0±0.4	6.3±0.6	9.4±0.8	12.1±0.8	0.6±0.2	3.2±0.5	7.7±0.6	12.8±0.8	16.7±0.9
Nucleus accumbens											
Ringer	(7)	3.2±0.3	5.9±0.4	9.2±0.6	11.7±0.7	14.8±0.8	1.9±0.3	3.5±0.3	11.8±0.6	16.1±0.7	20.6±0.9
TRH	(7)	2.0±0.5	3.8±0.6	7.5±0.6	10.8±1.1	12.8±1.7	1.8±0.5	5.2±0.7	10.3±1.0	14.6±1.0	17.2±1.5
Substantia nigra											
Ringer	(7)	3.1±0.2	5.6±0.2	8.3±0.5	11.2±0.9	13.8±1.1	2.9±0.2	6.1±0.5	10.8±0.4	15.0±0.6	18.5±0.7
TRH	(7)	2.5±0.3	4.5±0.5	8.1±0.2	11.7±0.4	14.0±0.8	1.4±0.3	5.0±0.5	10.2±0.7	15.1±0.7	18.3±0.7
Globus pallidus											
Ringer	(4)	3.3±0.5	5.8±0.7	8.6±0.7	11.5±1.0	14.4±1.5	2.1±0.1	4.8±0.7	9.8±0.8	14.4±1.1	18.2±1.7
TRH	(4)	3.4±0.5	5.8±0.6	8.6±0.7	11.1±0.3	14.8±0.8	1.4±0.2	5.0±0.7	9.7±0.8	14.2±0.6	18.4±1.1
Amygdala											
Ringer	(6)	3.1±0.3	5.5±0.4	9.8±0.4	10.9±1.0	13.2±1.5	2.3±0.4	5.4±0.8	10.8±0.4	14.6±0.8	18.8±1.8
TRH	(6)	3.1±0.2	5.7±0.2	9.3±0.6	12.7±0.7	15.5±0.6	1.2±0.5	4.0±0.4	10.2±0.8	15.2±0.8	20.1±1.0

The number in parentheses next to treatment group refers to the number of animals per group.

sium concentration [33]. Miyamoto and Nagawa have recently demonstrated [21] that sniffing, grooming and preening may be mediated via the action of TRH on both the mesolimbic and nigro-striatal dopamine system, since these behaviors occurred with both peripheral injection of TRH and intra-caudate and intra-accumbens injection of TRH. It is also reported that lesions in the nuclei accumbens septi produced transient increase in food intake without affecting water consumption [19]. In the present report, we also found that the injection of TRH into the nucleus accumbens resulted in a significant suppression of food intake. And this was supported by the report [7] that the greatest concentration of high-affinity TRH-binding sites in calf and sheep extra-hypothalamic brain tissue was located in the nucleus accumbens and septum. From these results, it seems evident that nucleus accumbens is related to suppressive effect of TRH on food intake with the appearance of several behaviors.

It has been shown that the lateral hypothalamus plays an important role in controlling water intake [1, 9, 10]. Lesions of this region of the brain produce adipsia and stimulation, either electrical or chemical, elicits drinking [2, 11, 22]. The injection of TRH into the medial and lateral hypothalamus produced a suppression of water ingestion of the same magnitude as that seen in food intake.

As in the investigations of TRH-induced shaking [36] and TRH antagonism of pentobarbital-induced narcosis [4,17], we found that the most sensitive central site of action for anorectic and adipsic responses evoked by TRH can be localized to more medial brain regions. Lateral movement of the injection site away from the medial hypothalamus and into the lateral hypothalamus caused a dramatic reduction in the anorectic effectiveness of TRH but not its adipsic effectiveness. The result that the lateral hypothalamus was most sensitive to TRH in drinking behavior is an exception.

In summary, this study provides evidence allowing the formation of the conclusions. Firstly, data are presented demonstrating the high sensitivity of the medial hypothalamic area to TRH in anorexia and adipsia. Secondly, the fact that behaviors could be elicited with a small amount of TRH (8 nmol/hemisphere) supports the hypothesis that TRH may have a neurotransmitter or neuromodulator-like function in the central nervous system.

REFERENCES

1. Anand, B. K. and S. Dua. Hypothalamic control over water consumption in the rat. *Indian J. med. Res.* **46:** 426–430, 1958.

2. Andersson, B. and S. M. AcCann. The effect of hypothalamic lesions on the water intake of the dog. *Acta physiol. scand.* **35:** 312–320, 1956.

3. Breese, G. R., J. M. Cott, B. R. Cooper, A. J. Prange and M. A. Lipton. Antagonism 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. Brobeck, J. R., J. Tepperman and C. N. H. Long. Experimental hypothalamic hyperphagia in the albino rat. *Yale J. Biol. Med.* **15:** 831–853, 1943.
6. 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.
7. Burt, D. R. and S. H. Snyder. Thyrotropin releasing hormone (TRH): apparent receptor binding in the rat brain membranes. *Brain Res.* **93:** 309–328, 1975.
8. Cott, J. M., G. R. Breeze, B. R. Cooper, T. S. Barlow and A. J. Prange. Investigations into the mechanism of reduction of ethanol sleep by thyrotropin-releasing hormone (TRH). *J. Pharmac. exp. Ther.* **196:** 596–604, 1976.
9. Fisher, A. E. and J. N. Coury. Cholinergic tracinga central neural circuit underlying thirst drive. *Science* **138:** 691–693, 1962.
10. Greer, M. A. Suggestive evidence of a primary "drinking center" in the hypothalamus of the rat. *Proc. Soc. exp. Biol. Med.* **89:** 59–62, 1955.
11. Grossman, S. Eating or drinking elicited by direct adrenergic or cholinergic stimulation of the hypothalamus. *Science* **132:** 301–302, 1960.
12. Hökfelt, T., K. Fuxe, O. Johansson, S. Jeffcoate and N. White. Distribution of thyrotropin releasing hormone (TRH) in the central nervous system as revealed with immunohistochemistry. *Eur. J. Pharmac.* **34:** 389–392, 1975.
13. Hökfelt, T., K. Fuxe, O. Johansson, S. Jeffcoate and N. White. Thyrotropin releasing hormone (TRH)-containing nerve terminals in certain brain stem nuclei and in the spinal cord. *Neurosci. Lett.* **1:** 133–139, 1975.
14. Huang, Y. H. and G. J. Mogenson. Differential effects of incertal and hypothalamic lesions on food and water intake. *Expl Neurol.* **43:** 276–280, 1974.
15. Ishibashi, S., Y. Oomura and T. Okajima. Facilitatory and inhibitory effects of TRH on lateral hypothalamic and ventromedial neurons. *Physiol. Behav.* **22:** 785–787, 1979.
16. Jackson, I. M. O. and S. Reichlin. Thyrotropin releasing hormone (TRH): Distribution in hypothalamic and extrahypothalamic brain tissues of mammalian and submammalian chordates. *Endocrinology* **95:** 854–862, 1974.
17. Kalivas, P. W. and A. Horita. Thyrotropin-releasing hormone: central site of action in antagonism of pentobarbital narcosis. *Nature* **278:** 461–463, 1979.
18. Kohno, H., T. Sakurada, T. Suzuki, K. Kisara and H. Satoh. Changes in ingestive behavior, serum glucose and free fatty acids concentrations following the intracerebroventricular injection of spermine in rats. *Jap. J. Pharmac.* **31:** 863–873, 1981.
19. Lorens, S. A., J. P. Sorensen and J. A. Hervey. Lesions in the nuclei accumbens septi of the rat: Behavioral and neurochemical effects. *J. comp. physiol. Psychol.* **73:** 284–290, 1970.
20. Mailman, R. B., G. D. Frye, R. A. Mueller and G. R. Breeze. Thyrotropin-releasing hormone reversal of ethanol-induced decreases in cerebellar cGMP. *Nature* **272:** 832–833, 1978.
21. Miyamoto, M. and Y. Nagawa. Mesolimbic involvement in the locomotor stimulant action of thyrotropin-releasing hormone (TRH) in rats. *Eur. J. Pharmac.* **44:** 143–152, 1977.
22. Mogenson, G. J. and J. A. F. Stevenson. Drinking induced by electrical stimulation of the lateral hypothalamus. *Expl Neurol.* **17:** 119–127, 1967.
23. Morley, J. E. Extrahypothalamic thyrotropin releasing hormone (TRH)—Its distribution and its functions. *Life Sci.* **25:** 1539–1550, 1979.
24. Morley, J. E. and A. S. Levine. Thyrotropin releasing hormone (TRH) suppresses stress induced eating. *Life Sci.* **27:** 269–274, 1980.
25. Morley, J. E., A. S. Levine and C. Parsad. Histidyl-proline diketopiperazine decreases food intake in rats. *Brain Res.* **210:** 475–478, 1981.
26. Nicoll, R. A. Excitatory action of TRH on spinal motoneurones. *Nature* **265:** 242–243, 1977.
27. Nemeroff, C. B., P. T. Loosen, G. Bissette, P. J. Manberg, I. C. Wilson, M. A. Lipton and A. J. Prange. Pharmaco-behavioral effects of hypothalamic peptides in animals and men: Focus on thyrotropin-releasing hormone (TRH) and neurotensin. *Psychoneuroendocrinology* **3:** 279–310, 1979.
28. Oliver, C., R. L. Eskay, N. Ben-Jonathan and J. C. Porter. Distribution and concentration of TRH in rat brain. *Endocrinology* **96:** 540–546, 1974.
29. Pellegrino, L. J., A. S. Pellegrino and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain.* New York: Plenum Press, 1979.
30. Plotnikoff, N. P., A. J. Prange, G. R. Breese, M. S. Anderson and I. D. Wilson. Thyrotropin releasing hormone: Enhancement of DOPA activity by a hypothalamic hormone. *Science 178:* 417–418, 1972.
31. Renaud, L. P., J. B. Martin and P. Brazeau. Depressant activity of TRH, LH-RH and somatostatin on activity of central cortical neurones. *Nature* **255:** 233–235, 1975.
32. Schally, A., T. Redding, C. Bowers and J. Barrett. Isolation and properties of porcine thyrotropin-releasing hormone. *J. biol. Chem.* **244:** 4077–4088, 1969.
33. Schaeffer, J-M., J. Axelrod and M. J. Brownstein. Regional differences in dopamine-mediated release of TRH-like material from synaptosomes. *Brain Res.* **138:** 571–574, 1977.
34. Vijayan, E. and S. M. McCann. Suppression of feeding and drinking in rats following intraventricular injection of thyrotropin releasing hormone (TRH). *Endocrinology* **100:** 1727–1730, 1977.
35. Vogel, R. A., B. R. Cooper, T. S. Barlow, A. J. Prange, Jr., R. A. Mueller and G. R. Breese. Effects of thyrotropin-releasing hormone on locomotor activity, operant performance and ingestive behavior. *J. Pharmac. exp. Ther.* **208:** 161–168, 1979.
36. Wei, E., S. Sigel, H. Loh and E. L. Way. Thyrotropin-releasing hormone and shaking behaviour in rat. *Nature* **253:** 739–740, 1975.
37. Winokur, A. and R. D. Utiger. Thyrotropin-releasing hormone: Regional distribution in rat brain. *Science* **185:** 265–267, 1974.
38. Winters, A., R. Eskay and J. Porter. Concentration and distribution of TRH and LHRH in the human fetal brain. *J. clin. Endocr.* **39:** 960–963, 1974.
39. Yarbrough, G. G. Studies on the neuropharmacology of thyrotropin releasing hormone (TRH) and a new TRH analog. *Eur. J. Pharmac.* **48:** 19–27, 1978.
40. Youngblood, W. W., M. A. Lipton and J. S. Kizer. TRH-like immunoreactivity in urine, serum and extrahypothalamic brain: Non-identity with synthetic pyroglu-hist-pro-NH_2 (TRH). *Brain Res.* **151:** 99–116, 1978.