JCI The Journal of Clinical Investigation

Plasma thyrotropin-releasing hormone concentrations in the rat. Effect of thyroid excess and deficiency and cold exposure.

C H Emerson, R D Utiger

J Clin Invest. 1975;56(6):1564-1570. https://doi.org/10.1172/JCI108238.

Research Article

To investigate the physiology of thyrotropin-releasing hormone (TRH) secretion from hypothalamus and brain, a method for measurement of peripheral plasma TRH concentrations in rats was developed. Blood was collected in heparin and dimercaptopropanol containing [3H]TRH to determine recovery. The plasma was extracted with methanol and the redissolved dried methanol extracts applied to anti-TRH Sepharose columns. These columns bound greater than 80% of 125I-TRH applied and had a capacity in excess of 20 ng TRH. TRH was eluted from the anti-TRH Sepharose with acetic acid and quantitated by radioimmunoassay of the lyophilized acetic acid eluate. Mean recovery of unlabeled TRH was 44.7+/-6.1% (SD) and mean recovery of [3H]TRH was 44.0+/-4.0%. Mean plasma TRH concentrations, corrected for recovery, in plasma pools from eight groups of normal male rats (four to seven pools/experiment, five to seven rats/pool) ranged from 7 to 30 pg/ml (mean, 16). In experiments in which rats were given 5, 10, 15, 0r 50 mug thyroxine daily for 1 wk or in thyroidectomized rats, mean plasma TRH concentrations did not differ significantly from those of control animals sacrificed at the same time. In each experiment, four to seven plasma pools, each from five to seven rats, were processed from both control and experimental groups. No changes in plasma TRH concentrations were found in rats exposed to cold (4degreeC) for 30, […]

Plasma Thyrotropin-Releasing Hormone Concentrations in the Rat

EFFECT OF THYROID EXCESS AND DEFICIENCY AND COLD EXPOSURE

CHARLES H. EMERSON and ROBERT D. UTIGER

From the Department of Medicine, Abraham Lincoln School of Medicine, University of Illinois and Veterans Administration Hospital, Chicago, Illinois 60612, and the Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19174

ABSTRACT To investigate the physiology of thyrotropin-releasing hormone (TRH) secretion from hypothalamus and brain, a method for measurement of peripheral plasma TRH concentrations in rats was developed. Blood was collected in heparin and dimercaptopropanol containing [^{3}H]TRH to determine recovery. The plasma was extracted with methanol and the redissolved dried methanol extracts applied to anti-TRH Sepharose columns. These columns bound >80% of ^{125}I-TRH applied and had a capacity in excess of 20 ng TRH. TRH was eluted from the anti-TRH Sepharose with acetic acid and quantitated by radioimmunoassay of the lyophilized acetic acid eluate. Mean recovery of unlabeled TRH was 44.7±6.1% (SD) and mean recovery of [^{3}H]TRH was 44.0±4.0%. Mean plasma TRH concentrations, corrected for recovery, in plasma pools from eight groups of normal male rats (four to seven pools/experiment, five to seven rats/pool) ranged from 7 to 30 pg/ml (mean, 16). In experiments in which rats were given 5, 10, 15, or 50 μg thyroxine daily for 1 wk or in thyroidectomized rats, mean plasma TRH concentrations did not differ significantly from those of control animals sacrificed at the same time. In each experiment, four to seven plasma pools, each from five to seven rats, were processed from both control and experimental groups. No changes in plasma TRH concentrations were found in rats exposed to cold (4°C) for 30, 60, and 90-180 min. Significant increases in plasma thyrotropin (TSH) concentrations were found in all cold-exposed animals. These results provide no evidence that thyroid hormone excess or deficiency affects TRH secretion. If TRH secretion is responsible for cold-induced increases in plasma TSH concentrations, the increase in TRH secretion is of insufficient magnitude to alter peripheral plasma TRH concentrations.

INTRODUCTION

Thyrotropin-releasing hormone (TRH)[1] secreted into the hypothalamic hypophyseal portal system has an important role in the maintenance of pituitary thyrotropin (TSH) secretion. It is clear, however, that TRH is an important constituent of extrahypothalamic neural tissue as well as of the hypothalamus (1–3). The quantity of TRH present in hypothalamus and other regions of the brain is sufficiently large that its detection by radioimmunoassay, and verification of its presence as TRH by bioassay in some instances (2, 3), is relatively easy. Detection of TRH in plasma has proven more difficult, due in part to insufficient assay sensitivity and also to the presence in plasma of an enzyme(s) which very rapidly destroys TRH (4, 5). The significance of TRH in peripheral plasma and its relationship to TRH levels in hypophyseal portal plasma thus remains uncertain, as does the role, if any, of thyroid hormone in the regulation of TRH secretion. The detection of TRH in picogram quantities in human and rat plasma or blood has been described in several recent reports (6–8), and in one of these no effect of thyroid hormone excess or insufficiency was detected, but an increase after cold

Received for publication 19 May 1975 and in revised form 4 August 1975.

[1] *Abbreviations used in this paper:* TSH, thyrotropin; TRH, thyrotropin-releasing hormone; T4, thyroxine.

exposure, on rat peripheral blood TRH concentrations was found (7).

This report describes the development of a method for the extraction and concentration of TRH from plasma using the technique of affinity chromatography which allows detection of TRH in pooled plasma samples from normal rats. Plasma TRH concentrations in normal, thyroxine-treated, thyroidectomized, and cold-exposed rats have been determined.

METHODS

TRH radioimmunoassay. TRH measurements were made by radioimmunoassay as previously described (9) using synthetic TRH (Abbott Laboratories, North Chicago, Ill.) as standard. Assay sensitivity was 5–10 pg. Samples were assayed in at least two volumes, usually 200 and 100 μl, and all samples from an individual study were assayed at the same time. ^{125}I-TRH was repurified by Sephadex G-10 chromatography as previously described immediately before use in all assays and binding experiments (9).

[^{3}H]TRH. [^{3}H]TRH (40 Ci/mmol) was obtained from New England Nuclear, Boston, Mass., and repurified by cellulose thin-layer chromatography in an acetic acid-butanol-water system (1:4:5, vol/vol), as described by Nair et al. (4). The specific activity of the repurified [^{3}H]TRH was 44 Ci/mmol by radioimmunoassay.

Preparation of anti-TRH Sepharose columns. Rabbit anti-TRH IgG was prepared from anti-TRH serum prepared as described previously (10). 8 ml of IgG was prepared from 11.7 ml of anti-TRH serum by ammonium sulfate fractionation, followed by chromatography on DEAE-cellulose. The fraction eluted with 0.01 M sodium phosphate, pH 6.5, was dialyzed against water, lyophilized, and dissolved in 8 ml of 0.2 M $NaHCO_3$, pH 9. The protein content determined by the method of Lowry et al. (11) was 28 mg/ml.

Anti-TRH IgG was coupled to Sepharose 4B as described by Cuatrecasas and Anfinsen (12). 6 g of cyanogen bromide was used to activate 20 ml of Sepharose. The activated Sepharose was washed with 1,500 ml of 0.2 M sodium citrate, pH 6.5, and transferred to a closed 250-ml bottle. 200 ml of 0.2 M sodium citrate, pH 6.5, and 2 ml (56 mg) of anti-TRH IgG were added and the sample mixed slowly overnight at 5°C. The Sepharose and buffer were then transferred to a 2 × 10-cm column and the buffer collected in 10-ml fractions. No material absorbing at 280 nm was recovered in these fractions, and the fractions, after concentration to 1 ml by lyophilization, did not bind ^{125}I-TRH. These results indicated complete coupling of the anti-TRH IgG to the Sepharose. Individual Sepharose anti-TRH columns were made by transferring 1 ml of the coupled Sepharose to 0.7 × 10-cm columns.

^{125}I-TRH binding by anti-TRH-Sepharose was tested by applying ^{125}I-TRH (20,000 cpm) in 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.5, to individual 1-ml columns at 5° and 24°C. After washing with the same solution until radioactivity returned to background, the ^{125}I-TRH was eluted with varying concentrations of acetic acid.

Collection and treatment of plasma samples for recovery experiments. Experiments to estimate recovery during various stages of the extraction and concentration procedure were performed with human plasma since large quantities were readily available. The plasma were kept at room temperature for 2 h to destroy any endogenous TRH and then heated for 2 h at 60°C in a covered beaker to destroy the plasma enzyme(s) responsible for TRH inactivation (4, 5). Synthetic TRH was then added and the plasma extracted with two volumes of methanol. After centrifugation at 2,400 rpm for 40 min, the supernates were dried overnight at 60°C in an air stream. There was no greater recovery of TRH when shorter drying times or lower temperatures were used. The dried supernates were then dissolved in 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.5, in a volume equal to 50% of the initial plasma volume and applied to anti-TRH Sepharose columns at room temperature. After being washed with an additional 5-ml buffer, TRH was eluted with 8 ml of 1.5 M acetic acid into a lyophilization flask containing 1 ml 0.25% bovine serum albumin. The lyophilized samples were then dissolved in 1 ml H_2O for TRH radioimmunoassay. The anti-TRH Sepharose columns could be reutilized by washing with 20 ml of 1.5 M acetic acid and then with 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.5.

Collection and processing of blood from rats for plasma TRH measurement. Rat plasma pools were prepared by sacrificing five to seven animals by decapitation. Trunk blood was collected into a beaker on ice containing heparin and 30 mg 2,3-dimercaptopropanol, which has previously been shown to inhibit the destruction of TRH by plasma (13). The blood was centrifuged immediately after collection for 2 min at 5°C and all except 1.5 ml of plasma extracted with two volumes of methanol. The volume of plasma extracted ranged from 21 to 30 ml per pool in different experiments. The samples were then further processed as described above. In most experiments, 15 pg [^{3}H]TRH was placed in the blood collection vessel before bleeding and 0.3 ml was removed from the final concentrate for ^{3}H counting.

Calculation of plasma TRH concentrations. The TRH concentration in the original plasma pool was calculated according to the formula,

$$\text{TRH (pg/ml)} = \frac{\text{TRH-S (pg/ml)} \times 100}{\text{PV (ml)} \times \%[^3\text{H}]\text{TRH recovery}}.$$

TRH-S is the concentration of TRH in the final lyophilized sample in picograms per milliliter and PV is the milliliters of plasma collected. No attempt was made to correct for the 15 pg [^{3}H]TRH added or the loss of TRH in the 1.5 ml of plasma saved for assay of other hormones.

TSH assay. Rat serum TSH concentrations were measured by radioimmunoassay using the rat TSH assay kit kindly supplied by the Rat Pituitary Hormone Distribution Program of the National Institute of Arthritis, Metabolism, and Digestive Diseases. Separation of free and antibody-bound ^{125}I-TSH was done with the double antibody method. Assay sensitivity was 10–15 ng rat TSH-RP-1 and results are expressed in nanograms of this standard.

Thyroxine (T4) assay. Plasma T4 concentrations were measured by radioimmunoassay as described by Chopra (14) using a commercial anti-T4 serum (Endocrine Sciences, Tarzana, Calif.). Assay sensitivity was 1.6 μg/dl.

Statistical analysis. Results were analyzed using Student's *t* test. When samples contained undetectable hormone concentrations, as in the case of plasma TSH concentrations in certain of the pools from T4-treated rats, plasma T4 concentrations in some of the pools from thyroidectomized rats, or in certain of the TRH concentrates, the value for the sensitivity threshold of the assay was used to calculate the means.

In vivo studies. Male 200–220 g Sprague-Dawley rats were used. For a single experiment 60–90 rats were divided

into control and experimental groups and, within each group, into subgroups containing five to seven rats each. There were four to seven subgroups in both control and treatment groups in each experiment. On the day of sacrifice, blood from each subgroup was pooled and a single concentrate for TRH assay made. Experimental and control subgroups were sacrificed alternately. Plasma, 1.5 ml in amount, for TSH and T4 assay was removed before the addition of methanol.

Rats receiving T4 were injected subcutaneously daily for 7 days in doses ranging from 5 to 50 μg in different experiments. Control rats received the buffer vehicle at the same time. Animals were killed either on the day of final injection or the following day as indicated below. Thyroidectomized rats were killed 16 days after operation.

Intact, untreated rats were exposed to 5°C for up to 180 min in different experiments. Control rats were kept outside the cold chamber (CombiCold Lab, LKB Instruments, Inc., Rockville, Md.) at room temperature. Lighting conditions and noise levels were similar for both experimental and control groups.

RESULTS

Characterization of the anti-TRH Sepharose columns. Fig. 1 shows the pattern of radioactivity elution when ^{125}I-TRH in buffer was applied to 1.0-ml anti-TRH Sepharose columns. The columns were washed with buffer and then with acetic acid. ^{125}I-TRH binding at neutral pH was less at 5°C than at 24°C. Elution of ^{125}I-TRH with 1.5 M acetic acid also proceeded more slowly at 5°C than at 24°C. Elution was faster and more complete with 1.5 M than with 0.5 M acetic acid. As a result of these studies, all subsequent experiments were done at 24°C and 8.0 ml 1.5 M acetic acid was used for elution. Recovery of ^{125}I-TRH in the acetic acid eluate from anti-TRH Sepharose columns ranged from 74 to 90% (mean 81.7±5.2% [SD]) in 13 experiments in which the ^{125}I-TRH was applied in 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.5. When ^{125}I-TRH was added

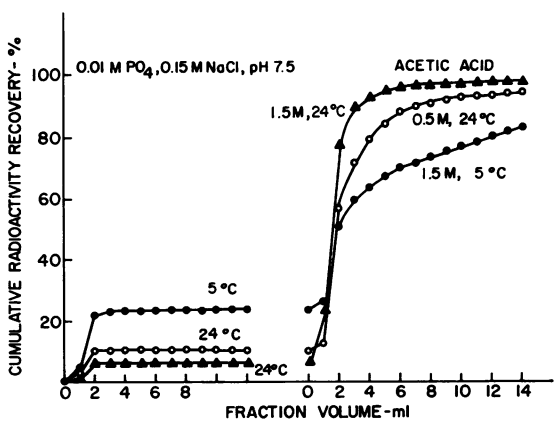

FIGURE 1 Binding and elution of ^{125}I-TRH from 1.0-ml columns of anti-TRH Sepharose. ^{125}I-TRH was applied in 0.01 M phosphate, 0.15 N NaCl, pH 7.5 at 5° and 24°C and eluted with varying concentrations of acetic acid.

TABLE I

Recovery of Unlabeled TRH Added to Plasma before Methanol Extraction and Affinity Chromatography

TRH added	n	Volume of plasma	TRH recovery
ng		*ml*	%
4	5	1	46.8±8.3*
4	5	5	38.2±9.6
4	5	10	51.8±4.1
4	5	20	41.8±5.2
Mean			44.7±6.1

* Mean±SD.

to methanol extracts of plasma dissolved in the same buffer, recovery of ^{125}I-TRH was similar, being 84.7± 2.0% (SD) in six experiments. The recovery of ^{125}I-TRH was similar (82.1-84.0%) when ^{125}I-TRH was applied to anti-TRH Sepharose columns with unlabeled TRH in quantities ranging from 1 to 20 ng.

Recovery experiments with plasma. Detailed studies of the recovery of TRH during the various stages of plasma extraction and concentration as well as of overall recovery in the procedure were carried out. Mean recovery of 0.25-16 ng TRH added to plasma and extracted with methanol was 68.1%, and the recovery was independent of the quantity of TRH added. When 2 ng TRH was added to 20 ml plasma from each of seven normal humans and the plasma was extracted with methanol and concentrated by affinity chromatography, the mean recovery was 41.3±13.5% (SD). Table I shows recovery data for the entire procedure when 4 ng TRH was added to 1-20 ml plasma. The mean TRH recovery was 44.7± 6.1% and did not decrease when larger plasma volumes were used. Recovery of [^{3}H]TRH added to plasma immediately before methanol extraction was 44.0±4.0%, a value similar to that for unlabeled TRH.

TRH in rat plasma. Table II shows the results of TRH determinations in plasma pools from normal rats. In each of the eight experiments, four to seven plasma pools were extracted and concentrated. Assay sensitivity was 50 pg/ml. TRH concentrations in the concentrates ranged from 73 to 304 pg/ml. The calculated mean TRH concentrations in these plasma pools from normal rats ranged from 7 to 30 pg/ml with an overall mean of 16±7 pg/ml (SD). The mean recovery of [^{3}H]TRH in these experiments was 37±9%, which was slightly less than that for [^{3}H]TRH or unlabeled TRH added to plasma before methanol extraction (see above) and probably reflects destruction of TRH during collection and centrifugation of the blood sample before methanol extraction.

These plasma concentrates, when assayed in 50-200-μl vol, produced dose-response lines parallel to that of

TABLE II

TRH Concentration in Plasma Pools from Normal Male Rats

Experiment	n*	[³H]TRH recovery	TRH in final concentrate	TRH in plasma pool
		%	*pg/ml*	*pg/ml*
1	6	52.0±10.7	192±79	16±5
2	6	25.0±6.8	96±24	11±1
3	4	43.6±4.1	73±25	7±3
4	7	29.6±2.0	98±28	12±4
5	5	42.6±3.0	118±21	13±2
6	5	35.6±4.3	304±108	30±9
7	6	37.8±6.7	198±133	22±15
8	6	31.1±4.4	85±36	14±8
Mean		37.0±9.0	145±80	16±7

* Number of pools, each containing plasma from five to seven rats.

unlabeled TRH in the radioimmunoassay (Fig. 2). In addition, the immunoreactivity of plasma concentrates was destroyed by incubation with 20% normal human serum at 37°C for 60 min (Table III).

T4 treatment. Plasma TRH concentrations in pools from rats receiving 5, 10, 15, or 50 μg L-T4 daily for 7 days are shown in Table IV. Mean plasma TRH concentrations in the normal animals varied from 7 to 47 pg/ml. In all four experiments the mean plasma TRH concentration in the T4-treated rats was not significantly different from that in the control group. In all the T4-treated groups the plasma T4 concentrations were significantly higher and the plasma TSH concentrations significantly lower than in the corresponding control group. The relatively small increase in plasma T4 concentrations in the animals given 10 and 50 μg T4 reflect the fact that these animals were killed 24 h after the last T4 dose, rather than 4 h after the last T4 dose as was the case in the rats receiving 5 and 15 μg T4 daily.

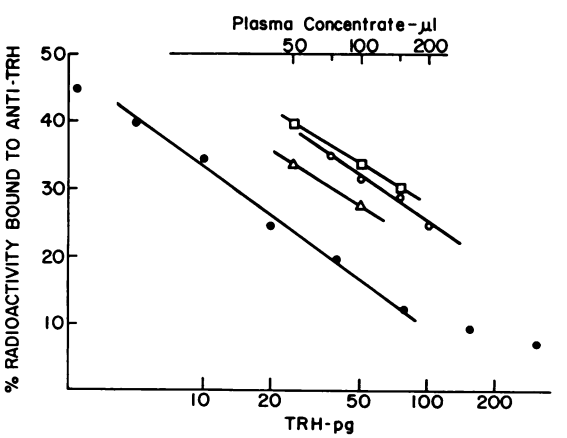

FIGURE 2 Comparison of the effect of unlabeled TRH and varying volumes of three TRH concentrates on the binding of ^{125}I-TRH to anti-TRH.

TABLE III

Effect of Human Plasma on the Immunoreactivity of Plasma Concentrates

	n	TRH*
		pg/ml
TRH concentrate + buffer‡	9	194±29
TRH concentrate + serum	9	<50

* Mean±SEM.

‡ Mixtures of four parts concentrate and one part assay buffer or serum were incubated at 37°C for 60 min.

Thyroidectomy. Table V shows the plasma TRH concentrations in thyroidectomized rats. In the two experiments the mean plasma TRH concentrations did not differ from those in the control group. Appropriate increases in plasma TSH and decreases in plasma T4 concentrations were found in the thyroidectomized rats in both experiments.

Cold exposure. Table VI shows the plasma TRH and TSH concentrations in rats exposed to 5°C for varying intervals in three separate experiments, carried out over a period of several months. In rats exposed to cold for 30 min, the mean plasma TRH concentration was 10±2 pg/ml (SEM), not significantly different from that in the control group, 14±3 pg/ml. While the mean plasma

TABLE IV

Plasma TRH, TSH, and T4 Concentrations in Rats Treated with Varying Doses of T4 for 7 Days

Treatment	n*	[³H]TRH recovery	Plasma TRH	Plasma TSH	Plasma T4
		%	*pg/ml*	*ng/ml*	*μg/dl*
Control	6	25.0±2.8	10±1	522±18	5.0±0.9
T4-5 μg/day	6	28.5±2.0	15±2	308±13	15.8±1.7
		NS	NS	$P < 0.001$	$P < 0.001$
Control‡	6	52.0±4.4	16±2	227±13	4.1±0.2
T4-10 μg/day	5	51.2±2.0	14±2	117±7	5.7±0.5
		NS	NS	$P < 0.001$	$P < 0.002$
Control	4	43.6±2.1	7±2	386±56	6.9±0.3
T4-15 μg/day	4	36.7±5.2	9±3	202±5	40.3±4.3
		NS	NS	$P < 0.001$	$P < 0.001$
Control‡	5	(37.2)§	47±14	276±37	4.4±0.21
T4-50 μg/day	5	(37.2)§	50±2	101±10	11.5±1.4
			NS	$P < 0.001$	$P < 0.001$

All Values are Mean±SEM.

* Number of plasma pools processed.

‡ Killed 24 h after last T4 dose. Animals receiving 5 and 15 μg T4/day were killed 4 h after the last T4 dose.

§ Recovery based on mean [³H]TRH recovery from entire study.

TABLE V

Plasma TRH, TSH, and T4 Concentrations (Mean±SEM) in Male Rats 16 Days after Thyroidectomy

Treatment	n*	[³H]TRH recovery	Plasma TRH	Plasma TSH	Plasma T4
		%	pg/ml	ng/ml	μg/dl
Control	7	29.6±0.8	12±2	810±309	4.0±0.1
Thyroidectomy	5	29.8±0.4	9±1	4,340±270	2.7±0.6
		NS	NS	$P < 0.001$	$P < 0.05$
Control	5	26.8±1.9	11±1	640±70	5.6±0.7
Thyroidectomy	5	22.2±2.4	11±2	3,710±770	2.3±0.3
		NS	NS	$P < 0.01$	$P < 0.01$

* Number of plasma pools processed.

TRH concentration was about 40% higher than that in control rats exposed to 5°C for 1 h (42±9 vs. 30±4 pg/ml), this difference was not statistically significant. In animals sacrificed between 90 and 180 min after the onset of cold exposure the mean plasma TRH concentration was slightly but not significantly lower than the control TRH concentration (17±4 vs. 22±6 pg/ml). The mean plasma TSH concentrations were higher in the cold-exposed rats than in the appropriate controls in all three experiments.

DISCUSSION

The results described here show that TRH can be extracted and concentrated from plasma with about 40% recovery of both [³H]TRH and unlabeled TRH. While unfortunately low, the recovery of TRH was consistent and independent of the quantity of TRH added to plasma. Taking into account losses during the procedure, the method yields an approximate 10-fold increase in TRH concentration with a starting plasma volume of 25 ml. It has been possible to detect a substance in pools of rat plasma which behaved like TRH in the TRH radioimmunoassay and which was destroyed by incubation with plasma. Based on these criteria, it is referred to as TRH in this paper. The method overcomes some of the problems of endogenous plasma TRH detection, such as inadequate assay sensitivity, destruction of TRH by plasma, and nonspecific effects of plasma in the TRH assay but is, of course, not as simple as would be desirable for wide application.

Plasma TRH concentrations in the individual pools from normal male rats ranged from 7 to 30 pg/ml, with a mean of 16 pg/ml. These values may be an overestimate. First, some of the [³H]TRH used to determine recovery could be trapped among sedimented erythrocytes. Secondly, since [³H]TRH was added at the beginning of the blood collection procedure, its recovery would more nearly reflect recovery of TRH from the first than from later blood collections if the BAL and cooling did not entirely prevent TRH destruction by plasma. Underrecovery of [³H]TRH would lead to higher calculated TRH concentrations since the extract TRH concentrations were corrected for 100% [³H]TRH recovery. The mass of [³H]TRH would add to the measured TRH concentration. However, this would be more than compensated by the 1.5 ml of plasma removed before methanol extraction for assay of other hormones. In any case, since the mass of [³H]TRH added was only 15 pg and the total amount of TRH in the concentrates of the normal plasma pools ranged from 168 to 840 pg, the error introduced by the [³H]TRH mass was very small.

At the present time there is very little published data regarding peripheral plasma TRH concentrations in the rat. TRH biological activity was found in peripheral plasma from cold-exposed hypophysectomized-thyroidectomized rats but quantitation was not possible (15). In a preliminary report, Oliver and co-workers found peripheral plasma TRH concentrations of 25-250 pg/ml in normal rats, measured by direct radioimmunoassay (6). Jackson and Reichlin reported a mean peripheral plasma TRH concentration of 13 pg/ml using an ethanol extraction and concentration method (16). Montoya et al. reported that TRH concentrations in whole blood from normal rats averaged 40 pg/ml (7). The TRH was recovered by precipitation of whole blood with $Ba(OH)_2$-$ZnSO_4$ reagent followed by affinity chromatography, and quantitation was by radioimmunoassay. The variability of these results undoubtedly reflects different methodology and problems of nonspecific effects of serum and in vitro TRH destruction.

At the present time it is unknown whether TRH in peripheral plasma is influenced by, or parallels, changes in TRH concentration in hypophyseal portal plasma. In view of the fact that there is considerably more TRH in

TABLE VI

Plasma TRH and TSH Concentrations (Mean±SEM) in Normal Rats during Cold Exposure

Treatment	Time*	n‡	[³H]TRH recovery	Plasma TRH	Plasma TSH
	min		%	pg/ml	ng/ml
Control	30	6	31.1±4.4	14±3	506±24
Cold-exposed	30	5	26.8±7.1	10±2	714±51
				NS	$P < 0.02$
Control	60	5	35.6±4.3	30±4	659±81
Cold-exposed	60	5	34.6±5.3	42±9	909±108
				NS	$P < 0.05$
Control	90–180	6	37.8±6.7	22±6	502±46
Cold-exposed	90–180	6	37.5±8.3	17±4	986±125
				NS	$P < 0.01$

* Minutes of cold exposure.

‡ Number of plasma pools processed.

rat extrahypothalamic brain tissue than in the hypothalamus (1–3), there may be very little relationship between peripheral and hypophyseal portal plasma TRH concentrations. Nevertheless, it was felt that investigation of possible thyroid hormone effects on peripheral plasma TRH concentrations was worthwhile. Evidence concerning effects of thyroid hormone excess or insufficiency on hypothalamic TRH is contradictory (17–19). No change in extrahypothalamic TRH content in T4-treated rats was found in studies done in this laboratory (20). Montoya et al. found no changes in blood TRH concentrations in thyroidectomized rats rendered hyperthyroid by T4 injections when compared to normal controls. Jackson and co-workers reported increased urinary TRH excretion in T4-treated rats and decreased urinary TRH excretion in hypothyroid rats (21).

The results described here fail to demonstrate any effect of thyroid hormone deficiency or excess on peripheral plasma TRH concentrations in rats. These results, of course, do not prove that thyroid hormones do not alter hypothalamic TRH secretion, since the source(s) within the brain of the TRH measured in peripheral plasma is not known, and since secretion of TRH from the hypothalamus into the hypophyseal portal system may constitute only a minor fraction of total brain TRH secretion. Furthermore, changes in TRH secretion, accompanied by parallel changes in TRH degradation, could have resulted in the data obtained here.

It has been known for some time that increases in plasma TSH concentrations follow acute-cold exposure in the rat (22-24). Striking, acute increases in blood TRH concentrations after cold exposure were found by Montoya et al. (7). No changes in plasma TRH concentrations followed cold exposure in this study. The reasons for these differences are unclear. It seems reasonable to assume that the plasma TSH increase after cold exposure is mediated by TRH. However, it is possible that a quantity of TRH entering the hypothalamic-hypophyseal portal circulation sufficient to double plasma TSH concentrations might not significantly change peripheral plasma TRH concentrations.

ACKNOWLEDGMENTS

We thank Miss Kathleen Kelley and Miss Joyce Koziel for expert technical assistance. Mrs. Beth Fischer and Mrs. Linda Ciocci provided excellent secretarial help.

This work was supported by U. S. Public Health Service grants 5 RO1 AM 14039 and 5 TO1 AM 05649 and by the Veterans Administration.

REFERENCES

1. Winokur, A., and R. D. Utiger. 1974. Thyrotropin-releasing hormone: Regional distribution in rat brain. *Science (Wash. D. C.)*. **185**: 265–267.
2. Oliver, C., R. L. Eskay, N. Ben-Jonathan, and J. C. Porter. 1974. Distribution and concentration of TRH in the rat brain. *Endocrinology*. **95**: 540–546.
3. Jackson, I. M. D., and S. Reichlin. 1974. Thyrotropin-releasing hormone (TRH): Distribution in hypothalamic and extrahypothalamic brain tissues of mammalian and submammalian chordates. *Endocrinology*. **95**: 854–862.
4. Nair, R. M. G., T. W. Redding, and A. V. Schally. 1971. Site of inactivation of thyrotropin-releasing hormone by human plasma. *Biochemistry*. **10**: 3621–3624.
5. Bassiri, R. R., and R. D. Utiger. 1972. Serum inactivation of the immunological and biological activity of thyrotropin-releasing hormone (TRH). *Endocrinology*. **91**: 657–664.
6. Oliver, C., R. L. Eskay, R. S. Mical, and J. C. Porter. 1973. Radioimmunoassay for TRH and its determination in hypophysial portal and peripheral plasma of rats. Program of the 49th Annual Meeting of the American Thyroid Association. T-4. (Abstr.)
7. Montoya, E., M. J. Seibel, and J. F. Wilber. 1975. Thyrotropin-releasing hormone (secretory) and physiology: Studies by radioimmunoassay and affinity chromatography. *Endocrinology*. **96**: 1413–1418.
8. Oliver, C., J. P. Chavet, J-L. Codaccioni, and J. Vague. 1974. Radioimmunoassay of thyrotropin-releasing hormone (TRH) in human plasma and urine. *J. Clin. Endocrinol. Metab.* **39**: 406–409.
9. Bassiri, R. M., and R. D. Utiger. 1972. The preparation and specificity of antibody to thyrotropin releasing hormone. *Endocrinology*. **90**: 722–727.
10. Deutsch, H. F., and J. L. Fahey. 1967. Purification of antibody. *Methods Immunol. Immunochem.* **1** (Sect. 3.A.2 and 3.A.3): 315–332.
11. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with Folin phenol Reagent. *J. Biol. Chem.* **193**: 265–275.
12. Cuatrecasas, P., and C. B. Anfinsen. 1971. Affinity chromatography. *Methods Enzymol.* **22**: 345–378.
13. Bassiri, R. M., and R. D. Utiger. 1974. Thyrotropin-releasing hormone. *In* Methods of Hormone Radioimmunoassay. B. M. Jaffe and H. R. Behrman, editors. Academic Press, Inc., New York. 37–44.
14. Chopra, I. J. 1972. A radioimmunoassay for measurement of thyroxine in unextracted serum. *J. Clin. Endocrinol. Metab.* **34**: 938–947.
15. Redding, T. W., and A. V. Schally. 1969. Studies on the thyrotropin-releasing hormone (TRH). Activity in peripheral blood. *Proc. Soc. Exp. Biol. Med.* **131**: 420–425.
16. Jackson, I. M. D., and S. Reichlin. 1974. Thyrotropin releasing hormone (TRH): Distribution in the brain, blood and urine of the rat. *Life Sci.* **14**: 2259–2266.
17. Jackson, I. M. D., R. Gagel, P. Papapetrou, and S. Reichlin. 1974. Pituitary, hypothalamic and urinary thyrotropin releasing hormone (TRH) concentration in altered thyroid states of rat and man. *Clin. Res.* **22**: 342A. (Abstr.)
18. Reichlin, S., J. B. Martin, M. Mitnick, R. L. Boshans, Y. Grimm, J. Bollinger, J. Gordon, and J. Malacara. 1972. The hypothalamus in pituitarythyroid regulation. *Recent Prog. Horm. Res.* **28**: 229–277.
19. Bassiri, R. M., and Utiger, R. D. 1974. Thyrotropin-releasing hormone in the hypothalamus of the rat. *Endocrinology*. **94**: 188–197.
20. Winokur, A., and R. D. Utiger. 1975. Distribution of

thyrotropin-releasing hormone in rat brain. *In* Anatomic Neuroendocrinology. W. E. Stumpf and L. D. Grant, editors. Karger AG, Basel. In press.
21. Jackson, I. M. D., P. D. Papapetrou, and S. Reichlin. 1974. The metabolism and excretion of TRH in the rat in states of altered thyroid function. Program of the 50th Annual Meeting of the American Thyroid Association. T-1. (Abstr.)
22. D'Angelo, S. A. 1960. Hypothalamus and endocrine function in persistent estrous rats at low environmental temperature. *Am. J. Physiol.* **199**: 701–706.
23. Ducommun, P., Sakiz, E., and R. Guillemin. 1966. Dissociation of the acute secretions of thyrotropin and adrenocorticotropin. *Am. J. Physiol.* **210**: 1257–1259.
24. Hershman, J. M., D. G. Read, A. L. Bailey, V. D. Norman, and T. B. Gibson. 1970. Effect of cold exposure on serum thyrotropin. *J. Clin. Endocrinol. Metab.* **30**: 430–434.
25. Brownstein, M. J., M. Palkovits, J. M. Saavedra, R. M. Bassiri, and R. D. Utiger. 1974. Thyrotropin-releasing hormone in specific nuclei of rat brain. *Science (Wash. D. C.)*. **187**: 267–269.