RELEASE OF THYROID STIMULATING HORMONE FROM CHICK ANTERIOR PITUITARY GLANDS BY THYROTROPIN RELEASING HORMONE (TRH)[1].

W.R. BRENEMAN AND WALTER RATHKAMP

Waterman Institute and Department of Zoology[2], Indiana University, Bloomington, Indiana

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SUMMARY- Chicks two and ten days-of-age respond to a wide range of thyrotropin releasing hormone (TRH) dosages as measured by thyroid uptake of ^{32}P. The duration of hormone and ^{32}P action is important. Excellent responses were obtained with the injection of 1.0 μCi ^{32}P at one hour and TRH either at one or four hours before autopsy in both two-day and ten-day-old birds. The ^{32}P uptake in the thyroid glands was increased by doses of hormone which ranged from 40 nanograms to 125,000 nanograms and was bimodal. Analysis of the data when calculated using $\log^{10}$ of dose was best accomplished by the use of 5th-degree polynomial equations. It is suggested that the bimodal response is a result of a dual action of TRH. First, TRH initiates the release of stored TSH from the anterior pituitary; and second, TRH stimulates the secretion of newly synthesized TSH by the anterior pituitary.

INTRODUCTION

The report (1) that TRH did not stimulate the release of thyrotropin (TSH) from the anterior pituitary glands of chicks when measured by ^{125}I release was unexpected for two reasons: first, we have been administering TRH utilizing the ^{32}P assay procedure (2) as a method of determining TSH reserve in anterior-pituitary glands of chicks at different ages and have observed significant responses to TRH treatment; second, the failure of TRH to be effective in the chick would seem to imply that there are fundamental differences between avian and mmammalian thyroid physiology and biochemistry which seem unlikely especially since anterior pituitary glands in general are known to be responsive to TRH over a wide range of dosages (3,4). We are reporting here few experiments which are indicative of certain of the problems encountered in TRH assay in chicks and which demonstrate that TRH is effective in stimulating TSH release.

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[1]Pyroglutayml-histidyl-prolyl-amide, Calbiochem, Torrey Pines California

[2]Contribution #112 from the Waterman Institute and #901 from the Zoology Department of Indiana University.

The data likewise illustrate that thyroid responses to TRH indicate the presence of significant and rapid physiological and biochemical events in the anterior-pituitary gland of the chick.

MATERIALS AND METHODS

Single-comb White Leghorn chicks either two or ten days-of-age were injected subcutaneously with thyrotropin releasing hormone (TRH) at either one or four hours before autopsy. Carrier free ^{32}P was administered one hour before autopsy. All chicks in a given group were killed by cervical luxation within ninety seconds. Glands were weighed within twenty seconds after removal and radioactivity was counted on a Nuclear Chicago automatic gas-flow counter. The ^{32}P activity was expressed as counts-per-minute minus background divided by the square root of the wet-weight of the glands in milligrams $\frac{(CPM-BG)}{\sqrt{mg}}$. Analysis of variance, covariance analysis, and estimations of responses by means of polynomial regression equations indicated that the expression of the data in this manner gave relatively low co-efficients of variation with the greatest differentials in responses and maximum F-values.

RESULTS AND DISCUSSION

The experiment summarized in Table 1 was designed to determine an effective time duration for TRH action. It was important to establish this relationship because the half-life of TRH in rat blood has been reported to be only four minutes (5). Accordingly, ten-day-old chicks were given TRH 20 minutes, 40 minutes, 80 minutes, or 160 minutes before autopsy and 4.0 μCi ^{32}P was administered simultaneously with the hormone. Another series of birds received only the 4.0 μCi ^{32}P in the same chronological sequences as the hormone-treated chicks and served as controls for each time interval. A relatively high dosage of 20.0 μg of TRH was injected in order to ensure TSH release if possible and the radioactivity of the testes also was determined to provide a comparison for ^{32}P uptake in target-organs only indirectly affected by the TRH-TSH axis..

The maximum uptake of ^{32}P in the thyroid glands of control animals occurred at twenty minutes and declined steadily during the succeeding time periods.

TABLE 1: Responses of Ten-Day-Old Chick Thyroids and Testes to the Simultaneous Administration of 20.0 μg TRH and 4.0 μCi ^{32}P at Different Time Intervals.

	TRH plus ^{32}P	^{32}P ONLY
20 MIN.	105.41±5.7 93.3±2.9	110.4±4.6 98.9±3.7
40 MIN.	101.8±16.6 75.3±2.4	90.5±12.1 74.5±3.3
80 MIN.	98.8±5.1 75.3±2.2	88.6±2.6 74.6±1.3
60 MIN.	104.2±5.8 94.1±4.6	84.8±3.3 84.9±2.5

ANALYSIS OF VARIANCE

Thyroids

SOURCE	DF	MSQ	F
TIME	3	3,169.73	4.23*+
TRH	1	1,933.39	7.74**
INTERACTION	3	618.55	2.47(NS)
ERROR	88	249.73	

Testes

SOURCE	DF	MSQ	F
TIME	3	2,848.59	26.65**
TRH	1	64.68	0.60(NS)
INTERACTION	3	233.07	2.06(NS)
ERROR	88	108.51	

There were 12 birds in each group (N-96) and data are expressed as means ± standard error of $\frac{CPM-BG}{\sqrt{wgt}}$. Data for testes are underlined.

*+ = 0.25, ** = 0.01, **+ = 0.005, *** = 0.001 (NS)= Non Significant

THYROID: TRH at 40-80-160 MIN. vs CONTROL at 40-80-160 MIN., F=13.36***
TRH at 160 MIN. vs CONTROL at 160 MIN., F=9.05***

TESTES: TRH at 160 MIN. vs CONTROL at 160 MIN., F=4.69*

The uptake of ^{32}P was slightly less in the TRH treated animals at 20 minutes and at that time the mean was not significantly different from that of the control. There was a decline in ^{32}P activity after 20 minutes. The variations were somewhat erratic but more importantly the ^{32}P activity of the TRH treated glands was greater than that of the controls at 40, 80, and 160 minutes with the maximum difference occurring at 160 minutes. Analyses of variances in the table indicate the degree of significance of the factors. It is evident that after 20 minutes TRH does affect the uptake of ^{32}P by chick thyroids due to the release of TSH by the anterior-pituitary.

The control data for the testes likewise demonstrated a decline in radioactivity with increasing time following injection. A somewhat similar pulse and decline for chick testes has previously been described (6). The TRH increased testes counts at 160 minutes but this was significant at only the 0.05 level. This response may represent a secondary effect of thyroid stimulation but this possibility will require further analysis.

TABLE 2: Responses of Thyroid Glands of Two-Day-Old Chicks to Graded Doses of TRH and 1.0 μCi ^{32}P.

Series A. - One Hour Treatment

Dosages*	0.7**	1.6	2.3	3.0	3.7	4.4	5.1
Actual Means	25.9±1.1	28.7±1.4	33.0±1.1	29.3±1.3	31.8±1.0	36.9±1.7	30.4±1.6
Calculated	25.9 --	29.7(29.5)	32.4(33.0)	30.2(29.3)	31.1(31.8)	37.2(36.9)	30.4(30.4)

5^{o} Equation: $\bar{Y} = 63.98 - 115.99X + 120.26X^2 - 53.02X^3 + 10.47X^4 - 0.76X^5$
Without Controls: $\bar{Y} = -257.32 + 445.85X - 253.94X^2 + 66.24X^3 - 7.81X^4 + 0.33X^5$

Series B. - Four Hour Treatment

Dosages*	0.7**	0.9	1.6	2.3	3.0	3.7	4.4	5.1
Actual Means	25.0±1.2	31.1±1.8	35.9±1.8	41.3±2.4	32.6±1.3	34.4±2.0	31.0±1.2	37.0±3.3
Calculated	25.5 --	30.0(30.9)	37.8(37.0)	38.2(38.7)	35.5±(35.7)	32.4(31.9)	31.8(32.0)	36.9(36.9)

5^{o} Equation: $\bar{Y} = -1.59 + 54.88X - 26.96X^2 + 6.13X^3 - 0.77X^4 + 0.05X^5$
Without Controls: $\bar{Y} = 32.23 - 20.94X + 33.95X^2 - 16.27X^3 + 3.07X^4 - 0.20X^5$

* Log^{10} of dose in nanograms, ** Controls were arbitarily given a log^{10} value of 0.7.
Estimated values in parentheses were calculated omitting the control (0.7) from 5^{o} polymomial.

Hormone and ^{32}P were administered one hour before autopsy in Series A. Hormone was injected four hours before and the ^{32}P one hour before autopsy in Series B. There were 14 birds in each group. Data are expressed as mean plus or minus standard error.

The ^{32}P assay technique for thyrotropin (2) has demonstrated that the thyroid gland of one or two-day-old chicks is excellent for detection of small quantities of TSH. Two additional experiments, therefore, were designed in which birds were administered a wide range of dosages of TRH accompanied by only 1.0 $\mu Ci^{32}P$ and the duration of treatment was for either one hour or four hours. The data are summarized in Table 2.

The dosages of TRH (expressed as $\log^{10}$ dose of nanograms) demonstrated that responses were definitely bimodal. Peak responses occurred in the one-hour experiment at the 0.20 µg TRH and at 25.0 µg TRH dosages ($\log^{10}$ 2.3 and 4.4 respectively). It is difficult to ascertain the best method of incorpating the responses of the control birds in these data. The controls were given an arbitrary value of $\log^{10} = 0.7$ and the data also were calculated excluding the control values from the determinations. Since the ^{32}P responses were bimodal various polynomial equations were tested to see which one would provide the best fit for the actual data. It was found that fifth-degree polynomial equations afforded excellent estimates of values for both the one-hour and the four-hour experiments (7). The equations and calculated values are included in the table. When the controls were excluded (estimated values are shown in parentheses in the tables), there was an especially remarkable correlation between observed and estimated values. The F-value for lack of fit in the one-hour series was a very low 0.05.

It occurred to us that the failure to elicit a greater response at the 125.0 ug dosage might be a result of the inability of the thyroid glands to react due to a threshold response, i.e. because of the short time period of one hour for the duration of hormone action. A second series of chicks, therefore, was injected at four hours before autopsy with TRH and with ^{32}P at one hour before killing. Also a lower dose of hormone, 0.008 µg was added to the series. Once again, the responses were bimodal but the maximum responses occurred at the 20.0 µg and 125.0 µg dosages ($\log^{10}$ 2.3 and 5.1). The estimated values based on the fifth-degree polynomial again gave excellent correlations with the

observed values and when the control thyroid data were eliminated from the calculation the F-value for lack of fit was only 0.08.

Experiments of the type shown in Table 2 have been repeated several times with comparable results and, in addition, the administration of TRH to ten-day-old cockerels has elicited similar results. The interpretation of these results leads to the projection of interesting biochemical analyses of both the anterior-pituitary glands and the thyroid glands of chicks. Since it has been reported that increasing doses of TRH cause progressively greater release of TSH and, equally as significant, that TRH stimulates synthesis as well as release of TSH from the anterior pituitary (5), our hypothesis is that the bimodal nature of the responses we have observed in the two-day-old chicks is a reflection of these two phenomena: first, that the initial peak and valley reflect the activation and release of stored TSH and the second peak is a result of increased TSH secretion. Fortunately, this hypothesis is one which can be tested biochemically and investigations presently are being directed along this line.

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