TSH Secretory Responses to Prolonged Infusion of TRH in Hypothyroid Rats

HITOSHI IKEDA and MONTE A. GREER
Section of Endocrinology, Department of Medicine, Oregon Health Sciences University, Portland, Oregon 97201, U.S.A.

Abstract. Continuous infusion of 0.1–10 µg/ml of TRH was performed through chronic iv cannulas in unrestrained, unanesthetized hypothyroid rats. Infusion of a constant concentration of TRH induced a peak in the plasma TSH concentration within 5–15 min which declined to the baseline within 1 h. The refractory period lasted 20–40 min after stopping the continuous TRH infusion. A second burst of TSH secretion was induced by increasing the TRH concentration during the refractory period while TRH was being continuously infused. These data indicate that in hypothyroid rats TSH secretion rapidly becomes refractory to continuous exposure to the same concentration of TRH but is stimulated by a higher TRH concentration. This suggests there is heterogeneity in TSH secretory units, which may consist of a constellation of thyrotrophs or of discrete intracellular secretory units, each with a threshold of specific response to TRH.

Key words: TRH, TSH, Hypothyroidism, Rats, Intravenous Infusion.

hormone secretion from the cells. The in vitro system permits more precise study of the characteristics of dynamic response than is possible in vivo but it has many characteristics different from the in vivo situation.

The present studies were undertaken to examine the above phenomena with respect to the TSH response to TRH in vivo.

Materials and Methods

Adult female Simonsen Sprague-Dawley rats weighing 200–250 g were used in all experiments. Before their use in specific experiments, they were housed 3–4 animals per cage in air conditioned rooms maintained at 24 ± 1°C with lights on from 0600 to 1800 h. They were fed Purina rat chow and tap water ad lib.

In the first and second experiments, the animals were surgically thyroidectomized 2 or 6 months, respectively, before they were studied. In the other experiments, the animals were given 0.03% methimazole in their drinking water for 1-3 weeks before the study.

Chronic iv cannulas were applied as described by Brown and Hedge [10]. A silastic tube (Dow-Corning #602-135, ID 0.02 inches, OD 0.037 inches) was inserted through the external jugular vein placing the tip of the cannula just rostral to the heart. The other end of the tube was pulled through a puncture incision in the dorsal scalp. The tube was fixed to the adjacent tissues with a highly adhesive glue (Krazy Glue, Japan). After cannulation, each animal was housed in an individual standard metal waste basket with a sawdust floor. Food and water were available ad lib.

Infusion of TRH and blood sampling

On the morning after cannulation, TRH infusion was begun at 0900 h. Synthetic TRH (Sigma) diluted with sterile 0.9% saline was infused through the cannula, with a Holter peristaltic pump. The flow rate was approximately 100 µl/min.

In the first experiment, 1 µg/ml of TRH was infused for 5 h. In the second experiment, 0.1 µg/ml of TRH was perfused for the first 3 h, 1 µg/ml for the second 3 h, and 10 µg/ml for the last 2 h. In the third experiment, 0.1 µg/ml of TRH was infused during the first 24 h and 1 µg/ml for the last 2 h. In the fourth experiment, saline, 0.1 µg/ml TRH or 1 µg/ml TRH was infused during the first 2 h and 1 µg/ml or 10 µg/ml TRH for the last 2 h. In the fifth experiment, a bolus injection of 0.1 µg TRH/100 g BW was given at various intervals after infusion of 1 µg/ml TRH for 1 h. Blood samples were obtained immediately before and 10 min after the TRH bolus injection. 0.25 ml blood samples were taken at various intervals through the cannula used for infusion by interrupting the infusion. It took approximately 1 min to withdraw each sample into an heparinized 1 ml syringe which was kept at 4°C and centrifuged. Plasma samples were stored at −20°C until analyzed. The erythrocytes remaining after removal of the plasma were not suspended in saline and reinfused since preliminary studies indicated that this procedure caused a significant increase in the plasma corticosterone concentration in a high proportion of the animals, and we wished to minimize any stress which might occur during the study.

Hormone determinations and statistical analysis

Plasma TSH concentrations were determined by RIA with rat kits supplied by the National Pituitary Agency and the NIDDK, NIH. TSH concentrations were calculated as µU/ml based on the potency of the NIH rat standard supplied (rTSH-R-P-1) of 0.22 U/mg. The plasma corticosterone [11] and thyroxine [12] were measured by radioimmunoassay. The minimal limits of detectability for each hormone were: TSH, 10 µU/ml; corticosterone, 5 ng/ml; thyroxine, 0.1 µg/dl. The samples for each experiment were measured in a single assay to avoid interassay variation. Statistical analysis was made by the paired t-test in the first, second, third and fourth experiments and by Student's t-test in the fifth experiment.

Results

Changes in plasma TSH concentration during infusion of 1 µg/ml of TRH for 5 h (Exp 1)

The data are shown in Fig. 1. The plasma TSH concentration peaked 5 to 15 min after starting TRH and returned to the baseline in 1 h. No
further significant changes occurred in the plasma TSH concentration during the next 4 h of infusion. Plasma T4 remained unchanged at 0.3±0.2 µg/dl before the infusion and 0.2 ± 0.1 at the end of the 5-h infusion. Plasma corticosterone was initially at approximately 200 ng/ml (normal for female rats) and gradually declined to approximately 100 ng/ml during the course of the infusion, indicating the procedure was not stressful.

Changes in plasma TSH concentration during a stepwise increase in infused TRH concentration (Exp 2)

The data are shown in Fig 2. A significant increase in the plasma TSH concentration over the preceding values was induced within 15 min by infusion of 0.1 µg/ml of TRH. Plasma TSH declined by 1 h to levels statistically indistinguishable from the pre-TRH infusion values. A similar rise and fall in the plasma TSH concentration occurred when the infusion concentration was raised to 1 µg/ml. A qualitatively similar but statistically non-significant rise was induced by infusion of 10 µg/ml TRH. No significant change was induced in the plasma T4 concentration (0.6±0.3 µg/dl before infusion, 0.5±0.3 µg/dl 6 h after beginning infusion, 0.5±0.3 µg/dl 8 h after beginning infusion).

Changes in plasma TSH concentration during a 24-h infusion of 0.1 µg/ml followed by infusion of 1 µg/ml of TRH for 2 h (Exp 3)

The data are shown in Fig 3. Basal TSH concentrations were lower than in Experiments 1 and 2, since the animals were given methimazole for only 1 week instead of being thyroidectomized for at least 2 months. The pattern of the plasma TSH concentration was the same as in previous experiments. It had increased by 15 min after infusion of 0.1 µg/ml of TRH and returned to the baseline in 1 h. It remained at this level throughout 24 h of perfusion with the same concentration of TRH. Compared to the immediately preceding values, there was a significant rise in the plasma TSH concentration at 15 min after the concentration of TRH was raised to 1 µg/ml.

The plasma T4 concentration was 0.9±0.1 µg/dl at 0 h, 0.8±0.1 at 10 h, and 2.2±0.4 µg/dl at 23 h.
Comparison of effect of infusion of saline or a low-concentration of TRH on TSH response to a following infusion of a higher concentration of TRH (Exp 4)

The TSH response to an infusion of 1 µg/ml of TRH was significantly weaker in rats previously infused with 0.1 µg/ml TRH for the preceding 2 h than for those infused with saline. There was no detectable TSH response to infusion of 10 µg/ml of TRH in rats which had been infused for the previous 2 h with 1 µg/ml of TRH (Fig. 4).

Length of period of resistance to bolus TRH injection after stopping continuous TRH infusion (Exp 5)

Rats were given an acute iv bolus injection of 0.1 µg/100 g BW at various times after stopping continuous 1 h infusion of 1 µg/ml of TRH (Fig. 5). Bolus TRH injection induced a significant increase in the plasma TSH concentration in control rats. This response was abolished for at least 20 min after stopping the continuous TRH infusion but a normal response returned by 40 min.

Fig. 4. Comparison of the effect of prior saline, 0.1 or 1 µg/ml TRH infusion on the TSH response induced by 1 or 10 µg/ml TRH infusion. Each group consisted of 4 hypothyroid rats given 0.03% methimazole for 3 weeks. They were infused with saline (panel A and C), 0.1 µg/ml of TRH (panel B) or 1 µg/ml of TRH (panel D) for the preceding 2 h, then with 1 µg/ml of TRH (panel A and B) or 10 µg/ml of TRH (panel C and D) for the last 2 h. Each circle and its vertical line represent the mean and SEM, respectively.
Discussion

The present in vivo data confirm our previous in vitro findings with acutely dispersed pituitary cells [7]. Continuous exposure to a constant concentration of TRH caused the thyrotrophs to become resistant to that concentration of TRH within a few minutes. However, a further rise in the plasma TSH concentration occurred when a higher concentration of TRH was infused. The amplitude of the TSH secretory response to continuous TRH infusion was inversely proportional to the concentration of TRH infused previously. The period of resistance to constant exposure to TRH is relatively short. It was between 20 and 40 min in our present in vivo studies and 8–10 min in the previous in vitro experiments. The shorter refractory period in vitro may be related to the inability to obtain sharp cutoffs in the TRH concentration in vivo because of recirculation of TRH through the closed vascular system before it can be degraded.

The loss of the TSH secretory response of the thyrotrophs to TRH during continuous TRH infusion in our experiments cannot be explained by an inhibitory effect of thyroid hormone on the thyrotrophs, since the animals were hypothyroid and plasma thyroid hormones were at very low levels both before and after the TRH infusions. Nor can it be explained by exhaustion of thyrotroph TSH stores, since infusion of a higher concentration of TRH induced a further burst of response as long as the preceding injection had not been at the maximal level (1 µg/ml).

The additional secretory response to an increase in the TRH concentration in the continuous infusion and the inverse relation of the amplitude of TSH secretion to the concentration of TRH in the previous infusion support and confirm the concept advanced in our previous in vitro study report [7] that there are heterogeneous TSH secretion units, each with a specific threshold of response to TRH. However, the present data do not rule out the possibility that there may exist several mechanisms in the intracellular signal transmission process which respond to a stepwise increase in TRH concentrations in stepwise fashion and cause a stepwise increase in TSH secretion.

In relation to the view described above, it is useful to review the studies by other investigators. Though to our knowledge no report has been made with respect to thyrotrophs, there are many reports of heterogeneity in lactotrophs [13–16] and somatotrophs [13, 17]. Boockfor et al. [13] and Luque et al. [14] used a reverse hemolytic plaque assay to show heterogeneity in lactotrophs with respect to responsiveness to TRH [13] and to dopamine [14], respectively. Arita et al. [15, 16] recently examined secretion by single lactotrophs with a sequential cell immunoblot assay and demonstrated directly that there is a heterogeneity in lactotrophs regarding PRL responsiveness to both TRH [16] and dopamine [15]. Boockfor et al. [13] showed heterogeneity of somatotrophs, with respect to the basal secretion rate of growth hormone, and Perez et al. [17] have recently demonstrated with a new tissue-slice method, that the heterogeneity in the somatotrophs is related in part to their location in the pituitary gland. To our knowledge, no study has yet directly revealed
heterogeneity in the thyrotrophs, but it is possible that there are subpopulations among the thyrotrophs with variable responsiveness to hypothalamic secretagogues.

Another mechanism that might cause the phenomenon shown in the present experiments is "down regulation" of TRH receptors induced by a constant infusion of TRH. Hinkle et al. reported that TRH regulates the number of its own receptors in the GH3 strain of pituitary cells in culture [18]. Resistance of TSH secretion to prolonged infusion with a constant concentration of TRH may be explained by a loss of TRH receptors in the thyrotrophs. However, TSH secretion response to a bolus injection of TRH recovered in a relatively short time (20–40 min) after stopping a continuous TRH infusion for 1 h, while Hinkle et al. demonstrated that TRH down-regulation of its own receptor persists for up to four days [18]. A similar "stair-case" of secretion is seen during continuous in vitro infusion of dispersed pituitary cells with osmolar stimuli which cause high amplitude secretory bursts (Greer MA, Sato N, Wang X, unpublished). In this case there is no exposure to exogenous TRH. With regard to the possibility of in vivo "down-regulation" of TRH receptor, Mori et al. have reported that hypothalamic TRH does not participate in controlling the TRH receptors judging from the results showing that neither the hypothalamic deafferentation nor the electoric lesion of periventricular nuclei altered the number or affinity of pituitary TRH receptors in thyroidecomtized hypothyroid rats [19]. Therefore, it is unlikely that the cause of the phenomenon observed in the present studies can be attributed to the down-regulation of TRH receptors.

The rats were infused with fluid at a high rate of 6 ml/h. We preferred a lower rate, but this was not possible with the equipment available to us. However, the animals seemed able to excrete the added fluid without difficulty and this high rate of infusion apparently did not interfere with the effect of TRH on TSH secretion. For example, in Exp. 4, saline infusion for 2 h did not have any detectable effect on the rise in plasma TSH concentration following a subsequent infusion of 1 or 10 µg/ml of TRH. Furthermore, the normal levels of plasma corticosterone in Exp. 1 indicate that the infusion procedure itself did not produce a significant degree of stress.

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References


