Exposure of Thyroid Slices to Thyroid-Stimulating Hormone Induces Refractoriness of the Cyclic AMP System to Subsequent Hormone Stimulation

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ABSTRACT These studies evaluated the influence of an initial exposure of thyroid slices to thyroid-stimulating hormone (TSH) on the subsequent responsiveness to the hormone. Bovine thyroid slices were incubated with or without 50 mU/ml TSH for varying periods and then incubated in hormone-free medium for varying periods. Subsequently, slices were incubated for 20 min with 10 mM theophylline and with or without TSH. Cyclic AMP was measured after the third incubation. Phosphodiesterase and adenylate cylase were assayed in homogenates prepared from slices after the second incubation. In some experiments prostaglandin E_1, puromycin, thyroxine, and triiodothyronine and propylthiouracil were included in the media. In other experiments, low doses of TSH (1 and 10 mU/ml) were used instead of 50 mU/ml.

Slices previously exposed to TSH have decreased responsiveness of the adenylate cyclase-cyclic AMP system. Such refractoriness is hormone specific since initial exposure to prostaglandin E_1 decreases the subsequent response to this substance but not to TSH. Refractoriness to TSH develops only when the first incubation is at least 30 min. It is not reversed by 5 h of incubation without hormone. Incubation of thyroid slices with puromycin does not eliminate refractoriness. The decreased response to TSH cannot be explained by release of thyroxine, triiodothyronine, or iodide from the slices. Phosphodiesterase activity is not increased during the refractory period. The decreased cyclic AMP response to TSH is associated with diminished response of adenylate cyclase activity to the hormone. Guanosine triphosphate (1 mM) increased adenylate cyclase activity in both control and TSH treated tissue, but the effect was significantly less in the latter. Although with guanosine triphosphate, TSH increased adenylate cyclase activity in TSH treated tissue, the enzyme activity was still less than that present in control tissue incubated with guanosine triphosphate and TSH. NaF caused an equivalent stimulation of adenylate cyclase in both control and TSH treated tissue. These results suggest that the refractoriness represents an alteration in hormone binding or the coupling of the bound hormone to the adenylate cyclase activity rather than any modification of the catalytic site of the enzyme.

INTRODUCTION

The effects of thyroid-stimulating hormone (TSH)[1] on thyroid metabolism are probably mediated by activation of the adenylate cyclase-cyclic AMP system (1). Although this system can initiate the diverse metabolic effects of TSH, less is known about the mechanisms by which the actions of TSH are terminated. Since TSH acutely does not increase cyclic AMP phosphodiesterase activity (1), this mechanism cannot account for the acute regulation of the hormone's effects on the thyroid. The development of refractoriness to hormonal stimulation after prior exposure to the hormone could provide a regulatory mechanism for control of hormone action. The existence of such refractoriness to hormonal stimulation has been reported in adipose tissue (2–4), cerebellum (5), ovary (6), liver (7), cerebral cortex (8), and lung fibroblasts (9). The present studies were done to determine whether similar refractoriness of the adenylate cyclase-cyclic AMP system developed in the

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[1] *Abbreviations used in this paper:* GTP, guanosine triphosphate; PGE_1, prostaglandin E_1; T_3, triiodothyronine; T_4, thyroxine; TSH, thyroid-stimulating hormone.

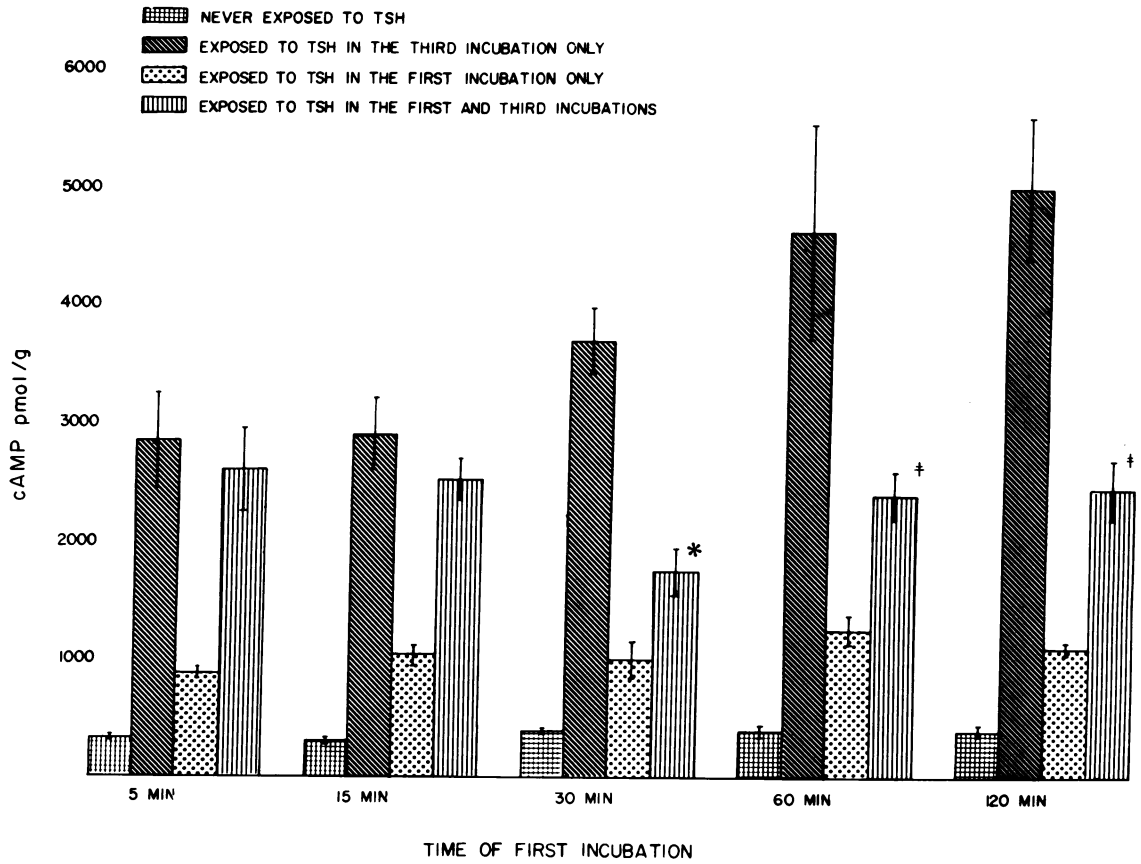

FIGURE 1 Effect of length of the first incubation on the subsequent cyclic AMP response to TSH. The second incubation was 120 min and the third incubation was 20 min. A single beef thyroid was used for the entire experiment. The values are the mean±SEM of triplicate slices. The TSH concentration was 50 mU/ml.
* $P < 0.01$ when compared to slices exposed to TSH in the third incubation only.
† $P < 0.05$ when compared to slices exposed to TSH in the third incubation only.
‡ $P < 0.02$ when compared to slices exposed to TSH in the third incubation only.

thyroid after exposure to TSH. Additional experiments were performed to elucidate the mechanism of such refractoriness.

METHODS

Experiments were performed primarily on beef thyroids; however, dog, lamb, and human glands were also studied. The results presented in the figures and tables were all obtained using beef thyroid glands. Beef and lamb thyroids were obtained from local abattoirs, transported to the laboratory in iced saline and used within 2 h of the animal's death. Human thyroids were obtained at the time of surgery for nonfunctioning nodules. Surrounding normal thyroid tissue was utilized. Dog thyroids were obtained from animals killed by exsanguination. Thyroid slices were made with a Stadie-Riggs microtome. Slices were initially incubated in a Dubnoff metabolic shaker at 37°C in 2 ml Krebs-Ringer bicarbonate buffer containing 1 mg/ml glucose, 1 mg/ml bovine albumin with or without TSH (50 mU/ml), or prostaglandin E_1 (PGE_1) (25 μg/ml) where appropriate. The gas phase was 95% O_2 and 5% CO_2. The time of the first incubation ranged from 5 to 120 min. After this first incubation, the slices were washed in an excess of 0.85% saline for 30 s, and a second incubation (30–300 min) was performed at 37°C in 2 ml Krebs-Ringer bicarbonate buffer with 1 mg/ml glucose. Slices were washed in 0.85% saline, blotted on filter paper, and weighed. They were transferred to fresh buffer containing 1 mg/ml glucose and albumin and 10 mM theophylline, and incubated at 37°C for 20 min. Half of the slices that had been incubated without TSH in the first incubation were again incubated in buffer without TSH, while the other half were exposed to TSH for the first time. Likewise, half of the slices incubated with TSH during the first incubation were now incubated without TSH while the other half were reexposed to TSH. After the third incubation the tissue was extracted in 0.4 ml of hot 0.05 M sodium acetate. After centrifugation, the supernate was assayed for cyclic AMP by a modification of the protein binding method (10). Adenylate cyclase and phosphodiesterase activities (1) were measured in homogenates prepared from slices after the second incubation. TSH stimulation of adenylate cyclase was examined by adding 1 or 10 mU of the hormone to the homogenate. To evaluate

phosphodiesterase activities with a low and a high K_m for cyclic AMP (11), the assay was done using both 1 and 100 μM.

Puromycin and propylthiouracil were used, respectively, to investigate the effects of protein synthesis and of iodide release into the medium on the development of refractoriness. Appropriate thyroid slices were incubated in Krebs-Ringer bicarbonate buffer with or without puromycin (150 μg/ml) or propylthiouracil (3 mM) for 15 min before commencing the first incubation. During the subsequent three incubations as outlined above, puromycin or propylthiouracil was again present. Experiments were also done with triiodothyronine (T_3) or thyroxine (T_4) (0.1 μM and 1 nM) in the medium during all three incubations to determine if this would induce refractoriness to TSH during the final incubation.

TSH (NIH-B6) was kindly provided by the National Institute of Arthritis, Metabolic, and Digestive Diseases. The sources of other materials has been reported (1).

RESULTS

Initial exposure of beef thyroid slices to 50 mU/ml TSH for at least 30 min significantly diminished the rise in cyclic AMP induced by reexposure to the same amount of hormone (Fig. 1). Despite an intervening incubation in the absence of TSH, slices exposed to TSH only during the first incubation had cyclic AMP values significantly higher than those in slices that had never been exposed to TSH. The higher cyclic AMP values reflected the interaction of TSH still present on the membrane from the first incubation and theophylline present in the third incubation (12). Nonetheless, compared to thyroid slices not incubated with TSH in the first incubation, the increase and absolute levels of cyclic AMP were significantly diminished in thyroid slices upon reexposure to TSH in the third incubation. Similar refractoriness was also found in dog, pig, lamb, and human thyroids (data not shown). The effect of varying the length of the second incubation on the development and persistence of the refractoriness to TSH was investigated (Fig. 2). The degree of unresponsiveness to TSH was not diminished even after a 5-h second in-

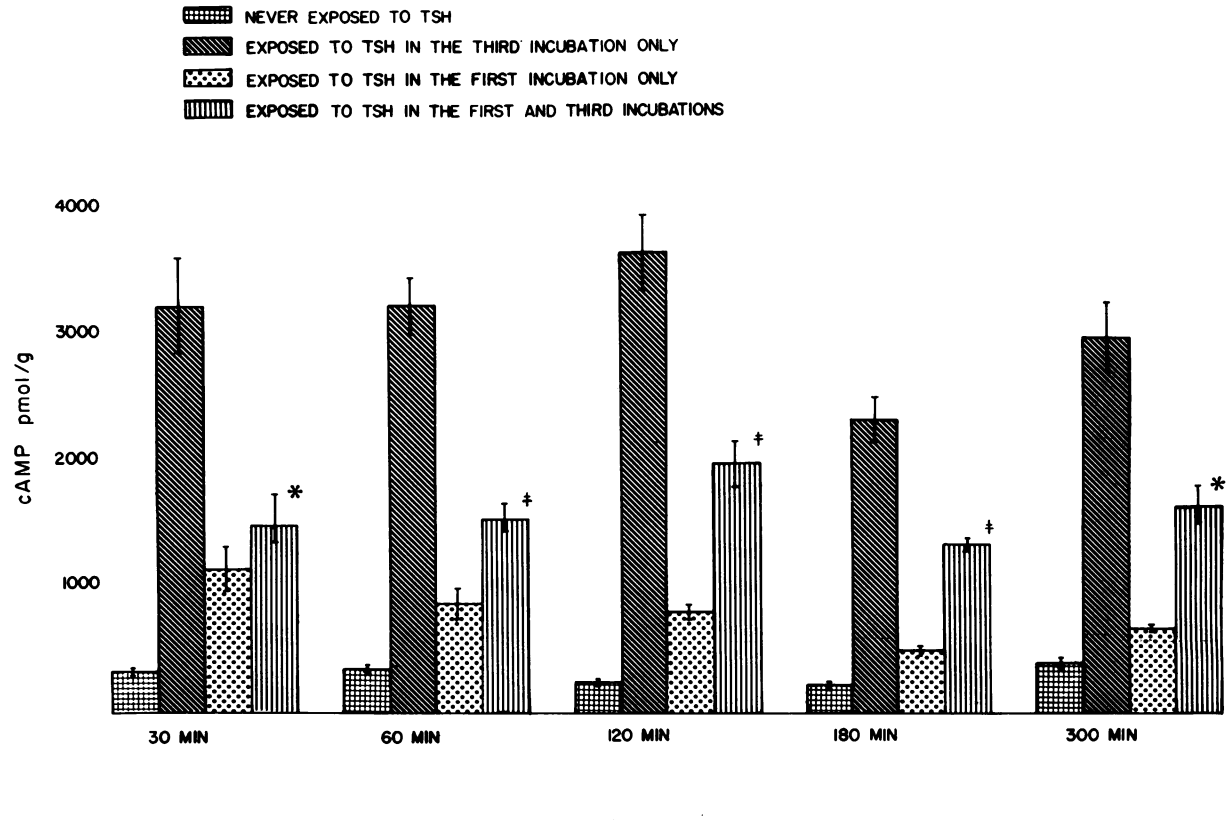

FIGURE 2 Effect of length of the second incubation on the subsequent cyclic AMP response to TSH. The first incubation was 120 min and the second incubation varied as indicated. The third incubation was 20 min. Different beef thyroid glands were used for each time. The values are the mean±SEM of triplicate slices. The TSH concentration was 50 mU/ml.
* $P < 0.02$ when compared to slices exposed to TSH in the third incubation only.
‡ $P < 0.01$ when compared to slices exposed to TSH in the third incubation only.

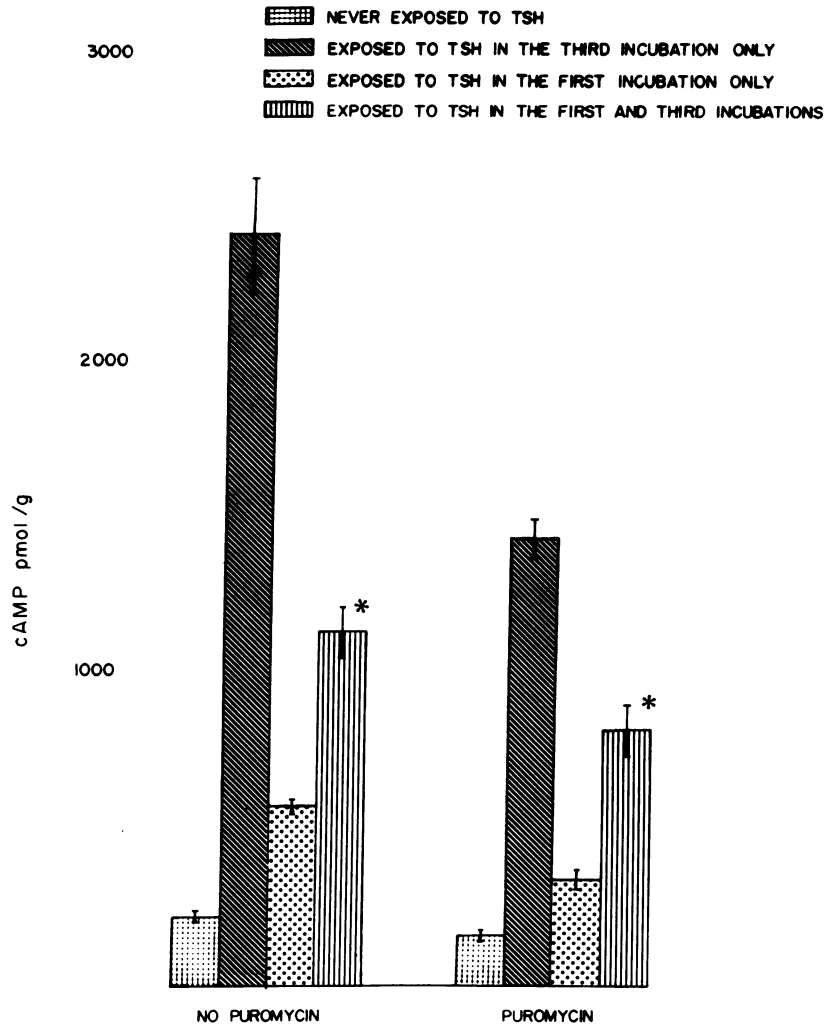

FIGURE 3 Failure of puromycin to modify refractoriness to TSH. Beef thyroid slices were incubated with or without puromycin (150 μg/ml) for 15 min before commencing the first incubation which was 120 min. All subsequent incubation media for slices initially exposed to puromycin also contained this substance. The second incubation was for 60 min and the third was for 20 min. The values represent the mean±SEM of triplicate slices. The TSH concentration was 50 mU/ml.
* $P < 0.01$ when compared to slices exposed to TSH in the third incubation only.

cubation in a hormone-free medium. Although puromycin decreased basal- and TSH-stimulated cyclic AMP concentrations somewhat, the refractoriness was not affected by incubation of thyroid slices with this material (Fig. 3). The reason for this reduction is not obvious, but it may represent a toxic effect of puromycin. In these experiments puromycin inhibited [^{14}C]leucine incorporation into protein by over 95% (data not shown). Since it has been reported that iodide (13, 14) and thyroid hormones (15–18) can inhibit the TSH activation of the adenylate cyclase-cyclic AMP system and since incubation of thyroid slices with TSH releases these substances into the medium (19), studies were done to determine whether they could be responsible for the refractoriness. The inhibition by iodide of the stimulation of the adenylate cyclase-cyclic AMP system by TSH is abolished by propylthiouracil or Tapazole which prevents organification of iodide (13, 14). These results of Fig. 4 indicate that the TSH-induced refractoriness is not due to release of iodide and subsequent organi-

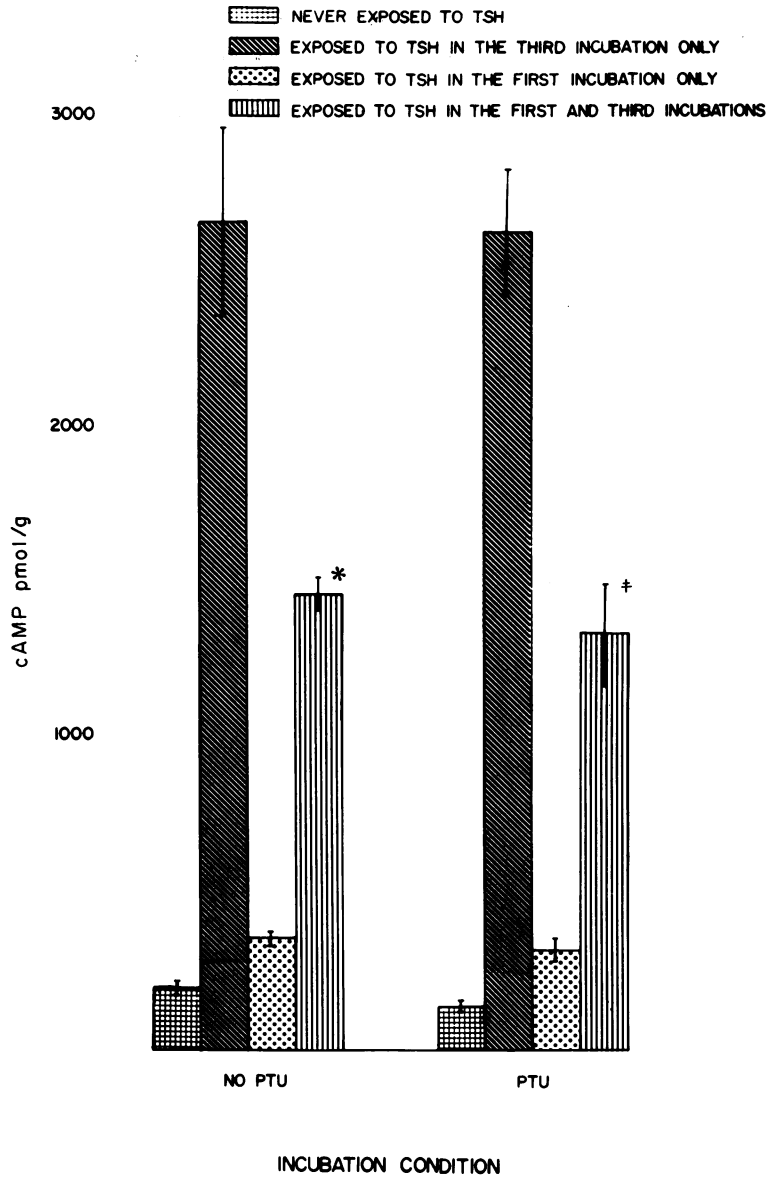

FIGURE 4 Failure of propylthiouracil (PTU) to abolish TSH refractoriness. Beef thyroid slices were incubated with or without (3 mM) PTU for 15 min before commencing the first incubation which was for 120 min. All subsequent incubation media for slices initially exposed to PTU also contained this substance. The second incubation was for 180 min and third for 20 min. The values represent the mean±SEM of triplicate slices. The TSH concentration was 50 mU/ml.

* $P < 0.02$ when compared to slices exposed to TSH in the third incubation only.

‡ $P < 0.01$ when compared to slices exposed to TSH in the third incubation only.

fication since incubation of slices with propylthiouracil did not modify hormone-induced unresponsiveness. Incubation of thyroid slices with T_4 or T_3 had minimal effects on the cyclic AMP response to TSH added during the third incubation compared to slices incubated in a similar fashion but without thyroid hormones (Fig. 5). In these experiments the thyroid hormones were present in the appropriate flasks during all three incubations. The refractoriness induced by TSH was not influenced by the presence of either T_4 or T_3 during all three incubations (data not shown).

The effect of submaximal doses of TSH on development of refractoriness was also examined (Table I). Incubation of thyroid slices with 1 and 10 mU/ml of

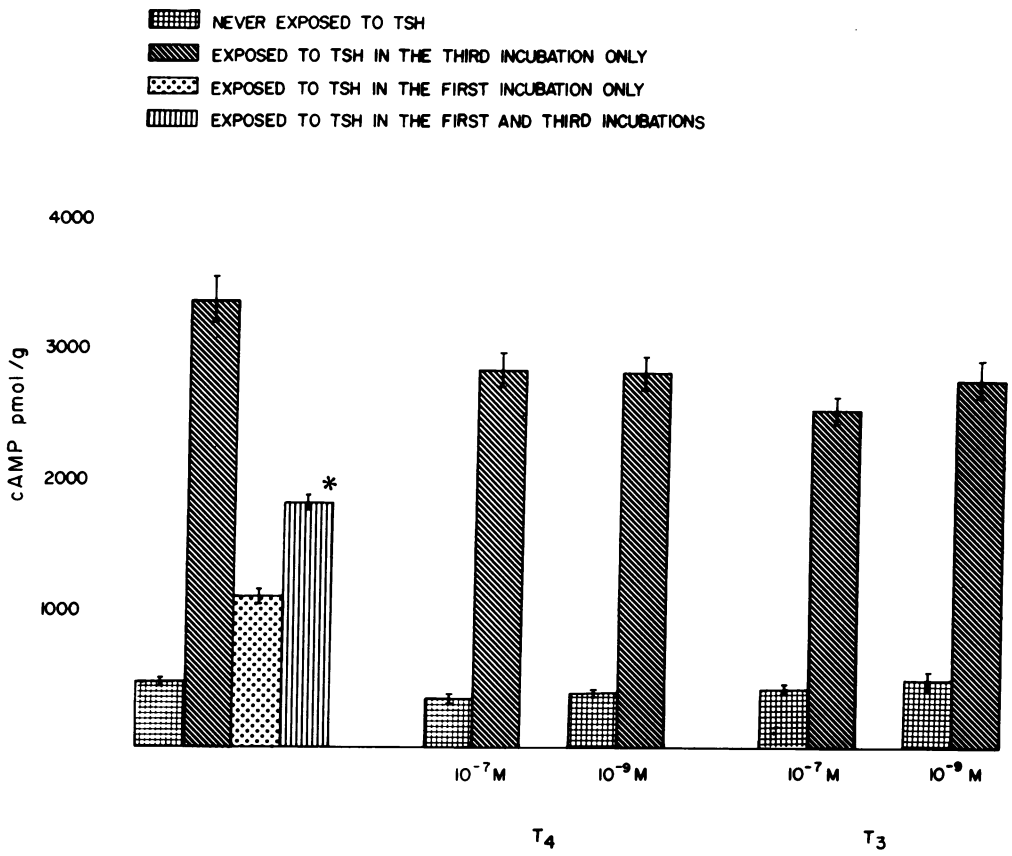

FIGURE 5 Failure of T_4 and T_3 to induce refractoriness to TSH. Beef thyroid slices were incubated with or without 0.1 μM or 1 nM T_4 or T_3 during all three incubations. The first and second incubations were for 120 min each while the third incubation was for 20 min. The values are the mean±SEM of triplicate slices. The TSH concentration was 50 mU/ml. In the presence and absence of 0.1 μM T_3, the effect of TSH added in the third incubation only was significantly less ($P < 0.05$) in the former slices.
* $P < 0.01$ when compared to slices exposed to TSH in the third incubation only.

TSH during the first incubation induced some unresponsiveness of the tissue to subsequent exposure to the same dose of hormone. However, the refractoriness induced by 1, but not 10 mU/ml of TSH in the first incubation could be overcome by utilizing 50 mU/ml TSH during the third incubation.

The specificity of the TSH-induced refractoriness was investigated using PGE_1, a substance that mimics many of the effects of TSH on the thyroid (1). Initial incubation of thyroid slices with PGE_1 (25 μg/ml) significantly inhibited the increase in cyclic AMP upon reexposure to PGE_1 but had no effect on the subsequent stimulation with TSH (Table II). The refractoriness induced by PGE_1 to subsequent stimulation by that substance could be reversed when the second incubation was prolonged from 2 to 5 h. In fact in the latter experiments, the response to PGE_1 appeared to be augmented by the prior incubation of thyroid slices with the substance in the first incubation. The reason for this increased responsiveness to PGE_1 is not apparent. The results suggest that initial exposure of thyroid slices to TSH did not induce refractoriness to PGE_1 added during the third incubation. Two factors may influence this conclusion. The stimulation induced by TSH greatly exceeds that due to PGE_1. In addition the persistent binding of TSH from the first incubation (12) increases the cyclic AMP concentration in the slices incubated in buffer and theophylline alone in the third incubation. This changing base line makes comparison difficult, but even so the increase caused by PGE_1 added only during the third incubation was as great in the thyroid slices initially incubated with TSH as that in slices incubated with buffer only. An attempt to overcome the effects of persistent TSH binding by increasing the second incubation from 2 to 5 h was only partially successful. Again, however, the increment of cyclic AMP in-

TABLE I

Effect of Submaximal Doses of TSH on Refractoriness

First incubation	Third incubation: TSH, *mU/ml* Control	1	10	50
	cAMP pmol/g			
Control	235±32	1,760±203	2,196±197	1,922±326
TSH, 1 mU/ml	265±37	1,153±32*	2,021±80	1,587±232
TSH, 10 mU/ml	552±20	858±24‡	1,291±78§	943±34‖
TSH, 50 mU/ml	715±97	—	—	840±33‖

Beef thyroid slices were first incubated for 120 min in buffer alone or with 1, 10, or 50 mU/ml TSH. The second incubation, in buffer alone, was 30 min. The third incubation of 20 min was performed as follows: slices from each of the four groups in the first incubation were incubated in buffer alone or with 1, 10, or 50 mU/ml TSH. The values represent the mean ±SEM of triplicate slices.

* $P < 0.05$ when compared to slices first exposed to 1 mU/ml of TSH in the third incubation.

‡ $P < 0.02$ when compared to slices first exposed to 1 mU/ml of TSH in the third incubation.

§ $P < 0.02$ when compared to slices first exposed to 10 mU/ml of TSH in the third incubation.

‖ $P < 0.05$ when compared to slices first exposed to 50 mU/ml of TSH in the third incubation.

duced by PGE_1 in the third incubation was certainly as great in the slices initially exposed to TSH as in the control slices.

The diminished cyclic AMP concentrations during reexposure of the thyroid slices to TSH could reflect augmented phosphodiesterase activity. D'Armiento et al. reported that increased cyclic AMP levels in fibroblasts were associated with augmentation of phosphodiesterase activity (20). Although TSH did not increase phosphodiesterase activity in thyroid slices during short incubations, the longer incubations utilized in the present studies might produce different results. The data in Table III indicate that prior exposure of thyroid slices to TSH and the attendant increase in cyclic AMP levels does not increase phosphodiesterase activity when measured just before the third incubation. This is true for both the high and low K_m enzyme activities.

TABLE II

Failure of Prostaglandin E_1 (PGE_1) to Induce Refractoriness to TSH

First incubation	Second incubation	Third incubation: Control	TSH	PGE_1
	h	*cAMP pmol/g*		
Control		584±52	6,978±99	1,963±138
TSH	2	2,310±198	4,385±164‡	4,157±104
PGE_1		671±48	6,893±186	1,364±100*
Control		518±67	6,344±349	1,219±139
TSH	5	1,053±63	3,604±108‡	2,428±182
PGE_1		600±7	7,717±212	1,813±113*

Beef thyroid slices were first incubated in buffer alone or with TSH (50 mU/ml/) or PGE_1 (25 μg/ml) for 120 min. They were then washed in saline and incubated for either 120 or 300 min in buffer. Subsequently slices from each of the three initial groups were incubated in buffer alone, TSH (50 mU/ml) or PGE_1 (25 μg/ml). A single beef thyroid gland was used for the entire experiment. Each value represents the mean±SEM of triplicate slices.

* $P < 0.05$ when compared to slices first exposed to PGE_1 during the third incubation.

‡ $P < 0.01$ when compared to slices first exposed to TSH during the third incubation.

TABLE III

Phosphodiesterase Activities during TSH Refractoriness

Assay substrate	First incubation: Control Phosphodiesterase Activity cAMP hydrolyzed	TSH
	pmol/mg per min	
Control	3.5±0.2	4.6±0.2
0.1 nmol cAMP	59.2±9.7	65.5±2.4
10 nmol cAMP	3,198±492	2,426±328

Beef thyroid slices were incubated with or without TSH (50 mU/ml) for 120 min, washed, and incubated in buffer for a second incubation of 30 min. Slices were then homogenized and assayed for phosphodiesterase activity. Each value represents the mean±SEM of triplicate determinations.

Refractoriness is associated with an inability of TSH to increase adenylate cyclase activity in homogenates of tissue previously exposed to the hormone (Table IV). TSH (1 and 10 mU) significantly increased adenylate cyclase activity in homogenates of thyroid slices which had not previously been exposed to the hormone. In contrast, homogenates from slices incubated with TSH during the first incubation did not respond with augmented adenylate cyclase activity when assayed with 1 or 10 mU of TSH. Refractoriness was not related to changes in the catalytic activity of the adenylate cyclase, since NaF (10 mM) stimulated enzyme activity equally in homogenates prepared from thyroid slices incubated with or without TSH during the initial incubation. Furthermore, the increase in adenylate cyclase activity in homogenates induced by TSH was not impaired by prior exposure of thyroid slices to PGE_1 (data not shown). Although guanosine triphosphate (GTP) (1 mM) augmented basal adenylate cyclase activity in both homogenates, enzyme activtiy was significantly less in the tissue that had previously been exposed to TSH. However, the percent stimulation was approximately the same in both tissues. While TSH did not increase adenylate cyclase activity in the TSH treated tissue, it did augment enzyme activity when GTP was also present. However, under these conditions enzyme activity was still not equivalent to that obtained in homogenates that had not previously been exposed to TSH, but the percent stimulation was similar.

TABLE IV

Failure of TSH to Stimulate Adenylate Cyclase Activity during Refractoriness to TSH

Substance present in assay	First incubation: Control	First incubation: TSH
	Adenylate cyclase activity [^{14}C]ATP incorporated into [^{14}C]cAMP	
	cpm/mg per 10 min	
Experiment 1		
Control	8.6±1.7	11.3±1.0
TSH, *1 mU/0.11 ml*	13.8±0.5*	11.7±0.8
TSH, *10 mU/0.11 ml*	23.3±1.8‡	12.6±1.4
NaF, *10 mM*	46.4±0.4‡	47.2±0.8‡
Experiment 2		
Control	30.8±0.6	26.3±1.9
TSH, *10 mU/0.11 ml*	50.5±0.9‡	28.9±0.3
GTP, *1 mM*	52.5±1.2‡	43.9±1.5‡§
GTP, *1 mM* + *TSH 10 mU/0.11 ml*	72.5±2.9‡**	59.6±2.9**§§
NaF, *10 mM*	145.8±1.5‡	141.4±2.1‡

Beef thyroid slices were incubated with or without TSH (50 mU/ml) for 120 min and washed. The second incubation was for 30 min. Slices were then homogenized and assayed for adenylate cyclase activity. In some assays 1 mM GTP was also included. Each value represents the mean ±SEM of triplicate determinations.

* $P < 0.05$ when compared to appropriate control.

‡ $P < 0.01$ when compared to appropriate control.

§ $P < 0.01$ when compared to 52.5±1.2.

** $P < 0.01$ when compared to the same homogenate incubated only with GTP 10^{-3} M.

§§ $P < 0.05$ when compared to 72.5±2.9.

DISCUSSION

Initial exposure of thyroid tissue from several species to TSH induces decreased responsiveness of the adenylate cyclase-cyclic AMP system when the tissue is re-exposed to the hormone. Induction of refractoriness requires at least 30 min of incubation of the tissue with TSH. Although binding of TSH requires less time (12) and its peak effect on cyclic AMP is evident within 10 min (1), the results suggest that refractoriness is not an immediate consequence of binding or elevated cyclic AMP levels but requires additional time for further metabolic changes to occur. The exact nature of such metabolic changes is not apparent, but they do not seem to be dependent upon new protein synthesis (Fig. 3). These results differ from those of DeVellis and Brooker who reported that actinomycin D or acetoxycycloheximide prevented refractoriness in rat glial tumor cells induced by norepinephrine (21). The refractoriness could not be attributed to release of iodide, and its subsequent organification, or thyroid hormone, (Figs. 4 and 5) although these substances can decrease the activation of adenylate cyclase by TSH (13–18). Prevention of organification of iodide abolishes such inhibition (13, 14) while in the present experiments, propylthiouracil did not modify refractoriness induced by TSH. These results do not exclude the possibility that inorganic iodide could cause refractoriness. In addition the presence of T_4 and T_3 in the buffer during all three incubations did not induce unresponsiveness of thyroid slices exposed to TSH only during the final incubation.

The refractoriness of thyroid, like several other tissues (2, 8, 9) differs from that reported for adipose tissue by Ho and Sutherland (4) since it was not reversed by repeated washing of the tissue. A second incubation without hormone for as long as 5 h did not abolish the decreased responsiveness to TSH indicating that the phenomenon is not easily nor rapidly reversed. Although PGE_1 produced diminished responsiveness to subsequent stimulation by this hormone after a second incubation of 2 h, it did not modify the response to TSH indicating some specificity for the induction of refractoriness. When the second incubation was prolonged to 5 h in the absence of PGE_1, the refractoriness of thyroid tissue to subsequent stimulation by this substance was no longer apparent. This contrasts to the results with TSH since refractoriness persisted even after a 5-h second incubation. This difference could reflect the more persistent binding of TSH compared to PGE_1 which has been previously demonstrated (12). The lack of hormonal cross reactivity in producing refractoriness is similar to that reported in guinea pig cerebral cortical slices (8), human astrocytoma cells (22), and human lung fibroblasts (9) but different than that found in adipose tissue (2). Such discrepancies could reflect differences in the underlying mechanism responsible for refractoriness.

The failure of TSH to stimulate adenylate cyclase activity in homogenates of thyroid slices previously incubated with TSH provides a basis for the refractoriness. This unresponsiveness to TSH stimulation could reflect several possibilities: (*a*) decreased binding of TSH to its receptors because they are either already occupied or have been altered by TSH from the first incubation, (*b*) the coupling mechanism between the hormone receptor and adenylate cyclase has been modified, or (*c*) inhibition of the catalytic activity of adenylate cyclase. The last possibility appears less likely since NaF and PGE_1 stimulated adenylate cyclase activity equally in homogenates from slices previously incubated with or without TSH. Furthermore, GTP also augmented adenylate cyclase activity in homogenates from tissue incubated initially with TSH although the absolute, but not the percentage, effect was somewhat less than that observed in control homogenates. The stimulation by GTP confirms the results previously reported by Wolff and Cook (23). Although the effect of this nucleotide has been attributed to an action at a nucleotide regulatory site, its exact relationship to the catalytic activity has not been elucidated (24). It has been

postulated that binding of GTP to hepatic adenylate cyclase results in a transition state of the enzyme which can then isomerize into a form with increased catalytic activity. This latter process is accelerated by glucagon. Although the results obtained with both GTP and TSH suggest that adenylate cyclase activity from tissue previously incubated with TSH is no longer refractory, interpretation of such experiments is somewhat difficult because of the different base lines with GTP alone. The amount of increase of activity induced by TSH and GTP compared to GTP alone in the two homogenates was similar, but the total enzyme activities were different. If GTP does diminish the refractoriness of adenylate cyclase to TSH, the mechanism is not apparent. Perhaps activation of the nucleotide regulatory site overcomes the alteration of either the TSH binding or the coupling step causing refractoriness. We have previously reported that GTP potentiation of stimulation of adenylate cyclase in thyroid plasma membranes by TSH was associated with decreased binding of TSH (25) while Moore and Wolff found no change in hormone binding (26). We have been unable to measure adequately binding of TSH to its receptors during refractoriness because the nonspecific binding using homogenates is too high to permit interpretation of the data. In adipose tissue an initial activation of adenylate cyclase is essential for induction of refractoriness since PGE_1 and nicotinic acid, both inhibitors of adenylate cyclase activity in adipose tissue, abolished refractoriness in adipose tissue induced by epinephrine (2).

The diminished cyclic AMP response to TSH was not due to increased phosphodiesterase activity. Assays were done for both the high and low K_m enzyme activities. Furthermore, refractoriness was demonstrated with theophylline in the buffer. Although inhibition of phosphodiesterase activity with isobutylmethylxanthine partially reversed refractoriness of cerebral cortical slices to biogenic amines (8), this did not seem to contribute to this phenomenon in rat glial tumor cells or adipose tissue (3, 21).

The possibility exists that some other, as yet unidentified, substance accounts for the inhibition of stimulation of the adenylate cyclase-cyclic AMP system by TSH. The extensive washing of the slices and the use of fresh medium excludes total release of such an inhibitor into the buffer. Evidence for such an inhibitory substance as an important component of the refractory phenomenon has been presented in several other systems. The feedback regulator from adipose tissue studied by Ho et al. was released into the medium (3). Fain (27) and Schwabe et al. (28) suggested that refractoriness to hormonal stimulation in adipose tissue might be mediated by release of adenosine into the medium. However, in brain slices, adenosine prevents refractoriness induced by biogenic amines (8). In the thyroid, effects of adenosine are quite complex. Although it stimulates cyclic AMP accumulation, it also partially inhibits the stimulation induced by TSH.[2]

The physiologic significance, if any, of such refractoriness to TSH stimulation remains unknown. It could provide a mechanism to modulate the action of TSH on the thyroid. Such mechanisms would be important since biologically active TSH persists bound to thyroid tissue (12). Although the major feedback loop controlling thyroid function is probably inhibition of TRH by thyroid hormones, other regulatory mechanisms may exist. The observation that chronic propylthiouracil treatment of animals with its attendant persistent elevation of TSH is still associated with goiter formation is not incompatible with the present demonstration of refractoriness to TSH. Since the refractoriness is not complete, there could still be expression of effects of TSH. However, this demonstration of refractoriness to TSH might be of no physiologic significance and may only reflect the results of in vitro incubations. This may well be the case in adipose tissue since Schimmel (2) reported that during the refractory period induced by epinephrine there was no diminution of glycerol release or the lipolytic response to the hormone despite significant reduction in generation of cyclic AMP.

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