EFFECTS OF THYROIDECTOMY AND TRIIODOTHYRONINE (T_3) ON THE SYNTHESIS AND RELEASE OF GROWTH HORMONE (GH) AND PROLACTIN

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Summary The rates of synthesis and release of growth hormone (GH), prolactin, and whole protein of the rat anterior pituitary were determined using an *in vitro* method.

1. Thyroidectomy caused a marked decrease in both synthesis and release of GH. Decreased synthesis was restored by a single injection of T_3 (20 μ g/100 g body weight) within 12 hr. Decreased GH release was also increased gradually by T_3 injection. The percentage release of GH was increased by thyroidectomy and the increase was depressed by T_3 injection.
2. Prolactin synthesis was increased by thyroidectomy, and the increased synthesis was suppressed as early as 3 hr after T_3 injection. Prolactin release was not affected by thyroidectomy. The percentage release decreased significantly after thyroidectomy.
3. Whole protein synthesis was increased by thyroidectomy and the increase was suppressed by T_3 injection. The whole protein release and the percentage release were not influenced by thyroidectomy and decreased significantly within 3 hr after T_3 injection.
4. Added *in vitro*, T_3 had no effect on synthesis of whole pituitary protein, but whole protein release was clearly suppressed at concentrations as low as 10^{-7} M.

These results indicated that thyroid hormone stimulated GH synthetic processes and depressed GH release processes. Prolactin synthetic processes were depressed by thyroid hormone. The mechanisms of the thyroid hormone action and the physiological significance of the facts observed are discussed.

It has been reported that *de novo* synthesis of whole protein in the rat anterior

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pituitary is affected by altered thyroid states. Thyroidectomy resulted in an increase of labeled amino acid incorporation into pituitary proteins, and thyroid hormone injection suppressed the increase (TONOUE and YAMAMOTO, 1967a, b, 1968b; LEE *et al.*, 1968; MATSUZAKI, 1970). These changes were correlated with altered thyrotropin (TSH) synthesis (TONOUE and YAMAMOTO, 1968a).

Concerning other pituitary hormones, reports have appeared on growth hormone (GH) content in the rat anterior pituitary. The GH content decreased in hypothyroid states induced either by thyroidectomy or by goitrogen treatment, and the content was subsequently normalized by administration of thyroid hormones (CONTOPOULOS and KONEFF, 1963; LEWIS *et al.*, 1965; SCHOOLEY *et al.*, 1966; ESCOBAR DEL REY *et al.*, 1968; SUZUKI and SHIBASAKI, 1970). Changes in prolactin content in the rat anterior pituitary under varied thyroid states have also been observed, though the results are not consistent (GROSVENOR, 1961; LEWIS *et al.*, 1965; SUZUKI and SHIBASAKI, 1970). These changes were inferred to show alterations in either synthesis or release of these hormones or both.

However, since the hormone content itself does not directly indicate hormone synthesis or release, it is necessary to determine directly the rates of synthesis and release, in order to clarify the hormone turnover in the anterior pituitary (YAMAMOTO *et al.*, 1970). Recent studies in our laboratory (SUZUKI and SHIBASAKI, 1970; MATSUZAKI, 1970) showed that GH synthesis in the rat anterior pituitary decreased markedly following thyroidectomy and that it returned to normal after T_4 injection.

A recently developed method (YAMAMOTO *et al.*, 1970), by which the rates of synthesis and release of GH and prolactin are simultaneously determined, has been effectively utilized in investigating these rates as influenced by age and sex (YAMAMOTO *et al.*, 1970), the estrous cycle (IEIRI *et al.*, 1971), and fasting (AKIKUSA, 1971). In the present study, the effects of thyroidectomy and T_3 injection on the synthesis and release of GH, prolactin, and whole pituitary protein were investigated employing this method. Some effects of T_3 added *in vitro* were also studied.

MATERIALS AND METHODS

Rats of the Wistar strain were purchased and kept in our animal room, in which the temperature was controlled at $25 \pm 1^\circ\text{C}$ and the light was turned off daily at 19:00 for 12 hr. The animals were fed on rat chow MF (Oriental Yeast Co.) and water *ad lib*. Except as otherwise stated, mature male rats of approximately 75 days of age were used.

In *in vivo* experiments, in which the effects of thyroidectomy and T_3 injection were observed, rats were surgically thyroidectomized 12 days before sacrifice. Rats of the control group were sham-operated at the same time. T_3 ($20 \mu\text{g}/100 \text{ g}$ body weight) was injected subcutaneously to thyroidectomized rats 3 or 12 hr before sacrifice. Animals were weighed before thyroidectomy and again before

sacrifice. Each excised anterior pituitary was immediately incubated with ¹⁴C-labeled leucine.

In *in vitro* experiments, in which the direct effects of T₃ were studied, normal or thyroidectomized male or female rats were used. Each anterior pituitary was preincubated for 2 hr with T₃ and then incubated with labeled leucine. The preincubation medium was Krebs-Ringer bicarbonate buffer, pH 7.4, containing 360 µg glucose and appropriate amounts of T₃.

All animals were decapitated with a guillotine between 9:30 and 10:30. Incubation with ¹⁴C-leucine was continued for 3 hr as has been described previously (IEIRI *et al.*, 1971). In *in vitro* experiments, the medium contained T₃ in appropriate amounts. After incubation, the pituitary and the incubation medium were separately treated for polyacrylamide gel disc electrophoresis as has been described previously (YAMAMOTO *et al.*, 1970). After the pituitary proteins were separated in the gel column, the protein bands were stained and gel blocks that contained GH or prolactin were dissected in approximately 4 mm thickness. Each gel block was soaked in 3 ml of glacial acetic acid containing 0.25% phosphotungstic acid for 3 hr, employing glass vials with gentle shaking. The soaking solution was replenished after 1 hr. The second soaking was carried out in 3 ml of a mixture of Cellosolve (ethylene glycol monoethyl ether) and glacial acetic acid (1:1, v/v) for 3 hr. By these successive soakings, the free labeled leucine contaminating the gel block was removed, and, at the same time, the gel block became completely transparent to scintillation and the radioactivity became countable.

The synthesis and release of whole protein in each anterior pituitary were also determined as follows: The remaining portion of each homogenate used for disc electrophoresis was mixed with a concentrated solution of separating gel to make the final concentration of acrylamide 7.5%. The mixture was polymerized in a small glass tube, the gel column taken out, and a block dissected from each column to be soaked as described above.

In order to measure the labeled leucine incorporated into proteins, each soaked gel block was put into 6 ml of toluene containing 0.3% PPO and 0.01% POPOP, and the radioactivity was counted in a Beckman liquid scintillation spectrometer, Model LS 200B. The counting efficiency was 90±1% in all samples.

From the radioactivity counts of labeled leucine, the rates of synthesis and release, and also the percentage release of GH, prolactin, and whole protein were calculated and expressed in cpm per rat or per 100 g body weight, following YAMAMOTO *et al.* (1970).

RESULTS

Effects of thyroidectomy on GH and prolactin

Body weight gain was less marked in thyroidectomized rats (Table 1). The

Table 1. Effects of thyroidectomy on synthesis and release of GH and prolactin.

Exp. No.	Body weight (g)		Synthesis (cpm) ^a		Release (cpm) ^b		Percentage release ^c	
	Initial	Final	Per rat	Per 100 g BW ^d	Per rat	Per 100 g BW		
GH								
1	{ Control	152±6 ^e	207±6	23586±3289	11581±1908	2421±233	1177±122	9.2±0.9
	{ Thyrex	151±3	180±5	13896±1222*	7728±673	1771±169*	978±78	13.0±1.2*
2	{ Control	222±3	272±5	30163±2138	11079±719	3948±236	1457±96	13.6±1.2
	{ Thyrex	226±9	248±8	21562±1042**	8740±513*	3047±130**	1230±34*	14.3±0.9
Prolactin								
1	{ Control	152±6 ^e	207±6	1360±226	673±132	230±21	112±10	19.0±3.0
	{ Thyrex	151±3	180±5	2183±210*	1204±95***	238±16	131±6	11.1±0.5*
2	{ Control	222±3	272±5	2767±259	1025±108	473±58	175±24	17.0±0.8
	{ Thyrex	272±9	248±8	3493±210	1420±106*	388±26	159±16	11.2±0.8***

Six rats comprised each experimental group. a; Radioactivity of ¹⁴C-leucine incorporated into GH or prolactin, both retained in the anterior pituitary and released into the medium. b; Radioactivity of ¹⁴C-leucine incorporated into GH or prolactin released into the medium. c; Release (cpm) per rat × 100/synthesis (cpm) per rat. d; Body weight. e; Mean ± standard error. *, **, *** indicate statistically significant differences from control values at the level of 5, 1, and 0.1%, respectively.

anterior pituitary weight per unit body weight was increased by thyroidectomy. The effects of thyroidectomy on synthesis and release of GH and prolactin are summarized in Table 1. Both GH synthesis and GH release per rat decreased significantly. The values per 100 g body weight (BW) also decreased, though the decrease was less marked because of the smaller body weight of the thyroidectomized animals. The percentage release of GH increased, the change being statistically significant in experiment 1.

The effects of thyroidectomy on synthesis and release of prolactin were studied using the same groups of rats. As shown in Table 1, prolactin synthesis (per rat or per 100 g BW) increased after thyroidectomy. Prolactin release did not appear to be affected by thyroidectomy. The percentage release of prolactin decreased.

Effects of T_3 supplement on GH and prolactin

Figure 1 shows the effects of T_3 injection on GH synthesis and release in thyroidectomized rats. Synthesis (per rat and per 100 g BW) began to increase 3 hr after T_3 injection, and the increase became statistically significant after 12 hr. Growth hormone release (per rat or per 100 g BW) also increased gradually with time after T_3 injection. The percentage release of GH decreased gradually.

Fig. 1. Effects of T_3 injection on synthesis and release of GH in thyroidectomized rats. Vertical bars indicate standard errors of the means. * indicates a statistically significant difference from the thyroidectomized (Thyrex) control at the level of 5%.

Fig. 2. Effects of T_3 injection on synthesis of prolactin in thyroidectomized rats. Symbols are the same as in Fig. 1.

Effects of T_3 injection on prolactin synthesis are shown in Fig. 2. Prolactin synthesis (per rat and per 100 g BW) decreased 3 hr after T_3 injection. The effects of T_3 injection on prolactin release and percentage release were not listed in Fig. 2, since the changes in these values were not consistent in repeated experiments, the effects being stimulatory in some and inhibitory in others.

Effects of thyroidectomy and T_3 supplement on whole protein

The results are summarized in Fig. 3. The whole protein synthesis (per rat or per 100 g BW) increased significantly after thyroidectomy, and the increased synthesis was suppressed rapidly to the normal level by T_3 injection, the changes being statistically significant after 12 hr.

Whole protein release (per rat or per 100 g BW) did not change notably after thyroidectomy and decreased significantly 3 hr and continued to decrease until 12 hr after T_3 injection. The percentage release changed similarly.

Effects of T_3 in vitro

When anterior pituitaries from normal mature male, thyroidectomized mature male, immature male, or immature female rats were incubated with T_3 *in vitro*, no consistent effects were observable on synthesis and release of GH and prolactin. Therefore, these data are not shown here.

Table 2 shows the *in vitro* effects of T_3 on synthesis and release of whole protein. Although no effects on synthesis were detectable, the release of the whole pituitary protein (per rat and per 100 g BW) was clearly depressed. The effects of T_3 were detectable at concentrations as low as $10^{-7}M$. The pituitaries from

Table 2. Effects of T₃ *in vitro* on synthesis and release of whole pituitary protein.

Exp. animals	Days after Birth	Body weight (g)	T ₃ concentration (M)	Synthesis (cpm)		Release (cpm)		Percentage release
				per rat	per 100 g BW	per rat	per 100 g BW	
Normal males	75	285 ± 6	0	66723 ± 2389	23682 ± 782	7915 ± 522	2848 ± 266	12.7 ± 1.1
		284 ± 6	10 ⁻⁷	71129 ± 4263	25070 ± 1587	7147 ± 293	2518 ± 100	10.1 ± 0.6
		281 ± 6	10 ⁻⁶	56613 ± 4551	20070 ± 1881	5301 ± 363**	1874 ± 138*	8.4 ± 1.2*
		280 ± 7	10 ⁻⁵	61824 ± 4796	23266 ± 2376	4054 ± 471**	1462 ± 195**	6.6 ± 1.0*
Thyroidectomized males	75	230 ± 10	0	60431 ± 2925	26832 ± 2377	4882 ± 423	2206 ± 223	8.1 ± 0.6
		230 ± 9	10 ⁻⁷	73860 ± 5057*	32421 ± 2664	3690 ± 150*	1615 ± 81*	5.3 ± 0.5**
		231 ± 9	10 ⁻⁶	67425 ± 5248	29014 ± 1382	3252 ± 281**	1403 ± 95**	4.9 ± 0.3***
		231 ± 9	10 ⁻⁵	67933 ± 4865	29605 ± 2334	2888 ± 79***	1263 ± 71**	4.3 ± 0.2***
Normal males	33	82 ± 2	0	59980 ± 4077	73203 ± 5020	6523 ± 543	7915 ± 567	11.1 ± 0.8
		83 ± 2	10 ⁻⁷	56065 ± 4700	68115 ± 5815	4936 ± 176*	5994 ± 286*	9.0 ± 0.4*
		82 ± 2	10 ⁻⁶	59783 ± 2216	73132 ± 3889	3872 ± 281**	4754 ± 428**	6.4 ± 0.3***
		84 ± 1	10 ⁻⁵	54168 ± 2531	64919 ± 4082	3239 ± 246***	3883 ± 349***	5.7 ± 0.4***
Normal females	33	68 ± 1	0	48639 ± 2312	72146 ± 4165	6601 ± 493	9764 ± 718	13.6 ± 0.9
		67 ± 1	10 ⁻⁷	48016 ± 1652	70163 ± 3483	4344 ± 555*	5810 ± 489*	9.0 ± 1.0**
		68 ± 2	10 ⁻⁶	48557 ± 2426	67896 ± 3606	4396 ± 385*	6355 ± 641**	8.0 ± 0.4***
		68 ± 2	10 ⁻⁵	46352 ± 2448	70540 ± 5637	3131 ± 218***	4612 ± 359***	7.4 ± 0.5***

Six rats comprised each experimental group. Symbols and abbreviations are the same as in Table 1.

Fig. 3. Effects of thyroidectomy and T_3 injection on synthesis and release of whole protein in the anterior pituitary. *, **, *** indicate statistically significant differences from the thyroidectomized (Thyrex) control at the levels of 5, 1, and 0.1%, respectively.

mature thyroidectomized male, immature male, and immature female rats appeared to be more sensitive to T_3 than the pituitaries from mature normal rats, since T_3 was effective in the latter only at concentrations above $10^{-6}M$. Similar depressive effects were also observable on the percentage release of whole protein.

DISCUSSION

The present study clearly demonstrated that GH synthesis is decreased markedly by thyroidectomy, while the normal level is restored by a single injection of T_3 of nearly physiological dose ($20 \mu g/100 g BW$) within 12 hr after the injection. These results indicate that the GH synthetic processes are stimulated and maintained by thyroid hormones. The present findings are supported by earlier reports from our laboratory. MATSUZAKI (1970), employing a method somewhat different from the present one, showed that GH synthesis depressed by thyroidectomy is restored by repeated injections of T_4 ($0.8 \mu g$ or more per 100 g BW). SUZUKI and SHIBASAKI (1970) reported that a single injection of T_4 ($20 \mu g$ /per 100 g BW) increased GH synthesis in thyroidectomized rats within 24 hr after the injection. In their experiment, GH synthesized and retained in the anterior pituitary was

measured, without taking into consideration GH synthesized and released into the medium. However, there is no substantial difference between their results and those of the present experiments, since only a small part (approximately 10%) of GH is released into the medium.

Growth hormone release is also depressed considerably by thyroidectomy, and the depression is eliminated by T_3 injection. The percentage release is slightly elevated by thyroidectomy, and the elevation is suppressed by T_3 injection. These results suggest that the GH release processes are more or less depressed by thyroid hormones.

The effects of thyroidectomy, that is, the depression of GH synthetic processes and the stimulation of GH release processes, readily explain the marked decrease in pituitary GH content observed after thyroidectomy (CONTOPOULOS and KONEFF, 1963; LEWIS *et al.*, 1965; SCHOOLEY *et al.*, 1966; ESCOBAR DEL REY *et al.*, 1968; SUZUKI and SHIBASAKI, 1970). Increased synthesis and decreased percentage release of GH by T_3 injection explain the increased pituitary GH content observed after thyroid hormone supplement (CONTOPOULOS and KONEFF, 1963; LEWIS *et al.*, 1965; SCHOOLEY *et al.*, 1966; ESCOBAR DEL REY *et al.*, 1968; SUZUKI and SHIBASAKI, 1970).

In contrast to GH synthesis described above, prolactin synthesis is increased by thyroidectomy, and the increase is suppressed by T_3 injection within 3 hr. These results suggest that the prolactin synthetic processes are stimulated by thyroidectomy and depressed by thyroid hormones. MATSUZAKI (1970) reported that prolactin synthesis did not change after thyroidectomy, and repeated injections of T_4 (20 μ g/100 g BW) for 5 days caused an increase in prolactin synthesis. As for prolactin release, the effects of thyroidectomy and of T_3 injection could not be confirmed in the present study, in spite of repeated experiments.

The changes in pituitary prolactin content reported so far are also inconsistent. GROSVENOR (1961) reported that repeated injections of T_4 into female rats caused an increase. LEWIS *et al.* (1965) did not find any notable change in male rats but found a significant decrease in female rats, in hypothyroid states. SUZUKI and SHIBASAKI (1970) found no change in prolactin content after thyroidectomy in female rats. A single injection of T_4 (20 μ g/per 100 g BW) caused either no change or an increase of the prolactin content.

The reasons for this general inconsistency in the results of studies on prolactin are not known. The activity of prolactin synthesis and release is very low in male rats (YAMAMOTO *et al.*, 1970), which may sometimes have led to obscure results. The activity level is high in female rats (YAMAMOTO *et al.*, 1970), but it fluctuates during the estrous cycle, being elevated specifically at estrus (IEIRI *et al.*, 1971). (This is the reason mature female rats were not used in the present study.) Further, prolactin appears to be more sensitively influenced than GH. For example, the change in prolactin synthesis induced by T_3 injection becomes significant after 3 hr, while that in GH synthesis after 12 hr. Prolactin synthesis and release are

significantly affected by cold exposure, which has no effect on GH (YAMAMOTO *et al.*, unpublished data). These observations indicate the need for more detailed studies on prolactin turnover.

As for the mechanisms of thyroid hormone action on the turnover of GH and prolactin in the anterior pituitary, a direct effect on the pituitary and an indirect effect through the hypothalamus should be considered. Since *in vitro* effects of T_3 on the synthesis and release of GH and prolactin are not detectable, it is tentatively assumed that thyroid hormones may act on the hypothalamus and indirectly affect the anterior pituitary by changing the action of the GH-releasing factor or the prolactin-inhibiting factor. NICOLL and MEITES (1963) reported that thyroid hormones stimulate prolactin release from the rat anterior pituitary cultured *in vitro* for 3 days. Whether this is due to the direct effect of the hormones on the prolactin release processes or to an indirect effect on the general activity of cultured cells remains to be clarified.

The physiological significance of the present findings is clear with GH, but not with prolactin. Retardation of the body growth of thyroidectomized animals can be ascribed to both the lack of thyroid hormones and the markedly reduced synthesis of GH produced by thyroidectomy. Since GH has no specific target organs and is utilized in most tissues, GH data expressed in terms of cpm per 100 g BW would be of more physiological significance (YAMAMOTO *et al.*, 1970). The depression percentage of GH synthesis per rat is approximately 30–40%, but the value per 100 g BW is 20–30% (Table 1). On the other hand, percentage release of GH is increased by thyroidectomy. These facts indicate that thyroidectomized animals can compensate to some extent for the markedly decreased GH synthesis.

Synthesis of whole protein in the anterior pituitary is stimulated by thyroidectomy, and this increase is depressed by T_3 injection. These results are consistent with the reports of TONOUE and YAMAMOTO (1967a, b; 1968b) and LEE *et al.* (1968). However these authors measured the radioactivity of labeled amino acid incorporated into pituitary protein, which were isolated by precipitation with trichloroacetic acid, excluding protein released into the medium. Their results were expressed in terms of specific radioactivity (cpm per mg protein). This mode of expression may not be suitable for evaluating the rate of protein synthesis when marked changes in protein content are expected. MATSUZAKI (1970) showed that the concentration of anterior pituitary protein is lowered by thyroidectomy and is restored by T_4 injection.

Later, however, MATSUZAKI (1970) showed that synthesis of whole protein expressed in terms of cpm per rat or per 100 g BW increases after thyroidectomy and that the increase is depressed by repeated injections of T_4 . His study, together with the present one, provides conclusive evidence for the stimulation by thyroidectomy and depression by thyroid hormones of whole protein synthesis in the anterior pituitary.

The stimulated synthesis of whole protein by thyroidectomy cannot be ascrib-

ed to changes in GH or prolactin. Synthesis of GH, which is the most prominent protein fraction in the anterior pituitary, is decreased by thyroidectomy. Prolactin synthesis is increased by thyroidectomy but the increase is too small to account for this stimulated synthesis. As was suggested before (TONOUE and YAMAMOTO, 1968b), increased TSH synthesis would be the main cause. Further, non-hormonal proteins may also contribute, since pituitary weight is increased by thyroidectomy.

Release of whole protein does not change after thyroidectomy, and T₃ injection causes a marked decrease. The fact that there is no change after thyroidectomy may be the result of the combined effects on various proteins, such as depressed GH release and stimulated TSH release. The marked decrease induced by T₃ injection may be due mainly to the decreased TSH release. In contrast to synthesis of whole protein, its release is depressed directly by T₃ *in vitro* at concentrations as low as 10⁻⁷M. This strongly suggests that thyroid hormones act directly on the anterior pituitary to depress TSH release, since the effect is not observable on GH or prolactin release *in vitro*.

TONOUE and YAMAMOTO (1967a, b, 1968b) first showed depression of protein synthesis by thyroid hormones in the anterior pituitary, which was in distinct contrast to their stimulating effects in the other tissues. The depressive effect developed within a few hours after the hormone injection, and the effect was also observable *in vitro* (TONOUE and YAMAMOTO, 1968b; LEE *et al.*, 1968). These findings led them to propose a new mechanism: Thyroid hormones act directly on the translation step of the protein synthetic processes in the anterior pituitary. The stimulatory effect of T₃ on GH synthesis (within 12 hr) and its depressive effect on prolactin synthesis (within 3 hr) also develop in a short period of time, though these effects are not observable *in vitro*. The action sites of T₃ in the GH and prolactin synthetic processes remain to be clarified.

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