SIMULTANEOUS ACCELERATION IN VIVO OF THE FORMATION OF THYROID RIBONUCLEIC ACID, PHOSPHOLIPID AND IODOPROTEIN BY THYROID-STIMULATING HORMONE

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The control by thyroid-stimulating hormone (TSH) of the growth and function of the thyroid gland is an excellent system for studying hormonal regulatory mechanisms because:
a) the hormone regulates the turnover of a unique iodinated protein, thyroglobulin (see Rall, Robbins & Lewallen, 1964) and
b) most of the effects of TSH in vivo can be reproduced in vitro in a variety of isolated thyroid preparations (Freinkel, 1964).

In a recent study, the enhancement of protein synthetic activity of target tissues by some growth and developmental hormones was found to be co-ordinated in time by an increased rate of synthesis of RNA and membrane phospholipids (Tata, 1966, 1967). TSH is known to exert a profound effect on phospholipid synthesis by thyroid tissue (Freinkel, 1964) and there is also some evidence of its influence on RNA levels (Creek, 1965). However, there is no information on how these two effects of the hormone would be temporally correlated to one of the main functions of the thyroid gland - the formation and iodination of thyroglobulin. We therefore decided to study the effect of TSH, as a function of time, on the synthesis of RNA, phospholipid and iodoprotein, both in vivo and in vitro. In this paper we report our results of the action of TSH in vivo on the guinea pig thyroid and in which it will be shown that the hormone causes a rapid and simultaneous acceleration of the formation of all three constituents.

MATERIALS AND METHODS: Male, albino guinea pigs (Mill Hill strain) weighing approximately 350 grams were used. Thyroid-stimulating hormone (0.074 i.u./mg.) from the Division of Biological Standards at this Institute was dissolved in 0.15 M-NaCl solution and administered

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as a single intramuscular injection. At the appropriate intervals after TSH and one to four hours before killing, each animal received an intraperitoneal injection of carrier-free [^{32}P]-orthophosphate and carrier-free ^{125}I in 0.5 ml. of 0.15 M-NaCl solution.

The thyroid glands from three or four similarly-treated animals were pooled and homogenized in 2 ml. of ice-cold 0.15 M-NaCl solution. Protein-bound ^{125}I was determined by precipitating a portion of each homogenate with ice-cold 20 per cent trichloracetic acid (Hocman et al., 1966). ^{32}P-labelled phospholipids and RNA were precipiated with 0.4 N-$HClO_4$ and the precipitate washed 2 - 3 times, with 0.4 N-$HClO_4$. Phospholipids were extracted from the washed precipitate with a 2:1 methanol-chloroform mixture twice at 20° and twice at 45° and then backwashed with 0.1 M-HCl and H_2O. The nucleic acids were then twice extracted from the residue with 2 M-NaCl solution at 90° and RNA separated from DNA according to the procedure of Tata and Widnell (1966). RNA was determined by the orcinol reaction (Ceriotti, 1955). The identity of the individual nucleotides was checked by their spectral properties after their separation according to the method of Katz and Comb (1963). The identity of the phospholipids was verified by the migration of their main components by thin-layer chromatography with chloroform:methanol:acetic acid:water (25:15:1.5:3) as the solvent (DeGraff et al., 1965). The phosphorus in the phospholipid fractions, and in some experiments the phosphorus in the ribonucleotide fraction, was determined by the molybdic acid procedure (Fiske and Subbarow, 1925). The ^{32}P and ^{125}I activity of the various fractions was determined in a Packard Tri-Carb liquid scintillation spectrometer.

RESULTS AND DISCUSSION: The time course of incorporation of ^{32}P into RNA and phospholipid and of ^{125}I into protein by the thyroid tissue of control and TSH-treated guinea pigs is shown in Table 1. The specific activity of the RNA and phospholipid fractions of control animals gradually increased with time after injection of isotope as did the total protein-bound ^{125}I. A single injection of 0.5 units of TSH six hours before killing increased the specific activity of RNA 5 to 15 times and that of phospholipid and total protein-bound ^{125}I approximately 3-fold.

In order to determine whether the TSH-induced increases in the specific activities of the three fractions studied were temporally co-ordinated, incorporation of ^{32}P into RNA and phospholipid and of ^{125}I into protein was examined one hour after giving the isotopes and at different times after TSH administration. The results of these experiments shown in Fig. 1 demonstrate firstly that the rate of incorporation of ^{32}P into RNA and phospholipid and of ^{125}I into iodoprotein were all considerably increased by TSH even one hour after

TABLE 1

INCORPORATION, AS A FUNCTION OF TIME, OF ^{32}P INTO RNA AND PHOSPHOLIPID AND ^{125}I INTO PROTEIN BY THYROID GLANDS OF GUINEA PIGS

Hours after ^{32}P and ^{125}I	RNA c.p.m./mg. RNA		Phospholipid c.p.m./mg.P		Protein-bound ^{125}I c.p.m./3 glands	
	Saline	TSH	Saline	TSH	Saline	TSH
1	184	899	12,623	38,100	12,090	46,615
2	200	2,980	15,680	42,400	29,220	71,100
4	668	7,410	30,000	93,600	-	-

Male guinea pigs were injected intramuscularly in groups of three with 0.15 M-NaCl solution or 0.5 units of thyroid-stimulating hormone six hours before killing. ^{32}P (50 μc./animal) and/or ^{125}I (3.75 μc./animal) was injected at the times indicated before killing. The thyroid glands from each group were pooled and the RNA, phospholipid and iodoprotein extracted and analyzed as described in the text.

administration of the hormone. Secondly, the increases in specific activities of RNA and phospholipid and in total protein-bound ^{125}I occurred simultaneously for all three components. It was not possible to show with one hour pulses of the isotopes that the enhancement of the labelling of RNA or phospholipid preceded that of thyroidal iodoprotein. TSH has been shown to affect the transport of a number of anions into thyroid tissue (Wolff, 1964). However, the increases in specific activities of RNA and phospholipid seen here in response to TSH cannot be explained on the basis of increased transport or availability of labelled phosphate since the total uptake (tissue content) of ^{32}P at any time was not greatly influenced by TSH treatment (Fig. 1).

The actual values for incorporation of ^{32}P into phospholipid and RNA and of ^{125}I into iodoprotein obtained in a typical experiment are shown in Table 2. That the increases in the rates of labelling of RNA and phospholipid caused by TSH indeed meant that their overall synthesis was increased was indicated by the net accumulation of RNA and to a lesser extent of phospholipid after hormone treatment. There was also a marked increase in the wet weight of the thyroid gland after TSH administration. It has been shown that the DNA content of thyroid tissue is not increased even 24 hours after TSH administration (Ekholm and Pantic, 1963).

More than 90 per cent of the ^{32}P in RNA hydrolysates was recovered in the four nucleotides in those experiments analyzed by ion-exchange chromatography.

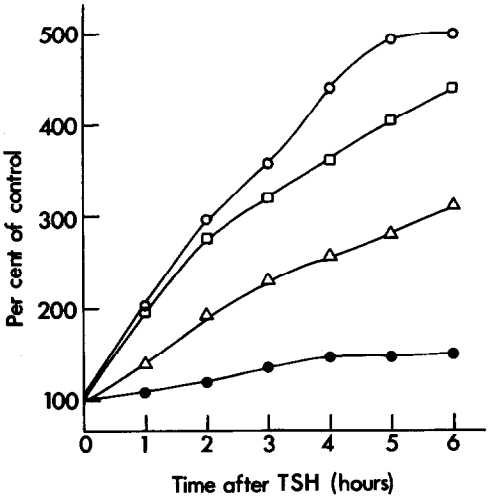

Fig. 1: Simultaneous stimulation by TSH of the incorporation of ^{32}P into RNA and phospholipid and of ^{125}I into protein of guinea pig thyroid. A 0.5 unit dose of TSH was given to each animal 1 to 6 hr. before killing. ^{32}P (50 μc.) and ^{125}I (3.75 μc.) were injected 1 hr. before killing. RNA, phospholipid and iodoprotein were extracted and analyzed as described in the text. Each point represents the average of at least 3 determinations (9 animals) and the values are expressed as the per cent of those of control animals (time 0). O, specific activity of RNA; Δ, specific activity of phospholipid; □, total protein-bound ^{125}I; ●, total uptake of ^{32}P.

It is, however, not yet possible to say whether or not the administration of TSH caused a shift in the distribution of ^{32}P into the bases. Much of the ^{32}P of phospholipids, after thin-layer chromatography, was recovered in phosphatidylcholine and phosphatidylethanolamine which are the main thyroid phospholipids (Freinkel, 1958). A part of the ^{125}I incorporated into thyroid protein was found to have the same electrophoretic mobility as purified pig thyroglobulin and was precipitated between 35 per cent and 45 per cent saturation with ammonium sulphate. It was in this protein fraction that TSH, 3 hr. after its administrati provoked the biggest increase in the incorporation of ^{125}I. A specific stimulat by TSH of incorporation of iodine into thyroglobulin has also been demonstrated in thyroid cell cultures (Raghupathy et al., 1965).

In order to establish the specificity of the action of TSH on the thyroid, portions of the livers from each group of animals were analyzed by the same procedures as used for the thyroid tissue. Significant amounts of protein-bound ^{125}I were not detected in the hepatic tissue of any of the animals studied. Further, the specific activities of the RNA and phospholipids of the liver tissue were not increased at early intervals of TSH treatment.

That TSH has a profound effect on phospholipid metabolism of the thyroid has been well established (Freinkel, 1964) and there is some indirect evidence that the hormone may affect RNA metabolism of the tissue (Creek, 1965; Begg and Munro, 1965). The important point of our findings is that the acute stimulation by TSH of the guinea pig thyroid in vivo involves a co-ordinated onset of accelerated synthesis of both phospholipid and RNA with one of the main biological actions of the hormone - iodination of protein and synthesis of thyroglobulin. We have also observed a similar simultaneous control in

TABLE 2

EFFECT OF TSH ON THE SYNTHESIS OF PHOSPHOLIPID, RNA AND IODOPROTEIN AND ON THE WET WEIGHT OF GUINEA PIG THYROID TISSUE

Hours after TSH	Phospho-lipid c.p.m./mg.P	RNA c.p.m./mg.P	PB^{125}I c.p.m.	Thyroid (average per gland) Wet wt. (mg.)	P-lipid (μg.)	RNA (μg.)
0	17,100	2,870	10,500	69	388	69.5
1	20,910	7,110	25,400	80	-	70.4
3	41,000	14,120	44,000	85	402	82.1
6	42,000	17,290	38,450	85	433	86.8

Three guinea pigs per group were injected with 50 μc. ^{32}P and 3.75 μg. ^{125}I 1 hr. before killing and 1 to 6 hr. after receiving an injection of 0.5 units of thyroid-stimulating hormone. The thyroid glands from each group were pooled and RNA, phospholipid and iodoprotein extracted and analyzed as described in the text. The values for thyroid weights are the average of 12 glands. The values for chemical RNA and phospholipid are the average of 4 separate determinations.

the action of TSH *in vitro* on monolayer pig and sheep thyroid cell cultures (unpublished results). This type of response of the target cell to the trophic hormone seems to be yet one more example of a co-ordinated control of RNA, phospholipid and protein synthesis observed in other systems of hormone-induced growth and development (Tata, 1967). It is not known whether a co-ordination of such diverse cellular activities in response to TSH only applies to iodination of thyroglobulin or could be extended to the other actions of the hormone; however, several reports have suggested that different biological actions of TSH (i.e. on iodide transport, glucose metabolism, synthesis of thyroglobulin, proteolysis, etc.) are mediated via different mechanisms (Freinkel, 1964; Rall, Robbins and Lewallen, 1964; Dumont, 1965). It would also be important to determine the cellular distribution pattern of the additional RNA, phospholipid and iodinated protein formed, especially with regard to their association with cytoplasmic membranes, under the influence of TSH on the thyroid cell.

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