Comparative Effects of TSH and of Insulin on Fetal Rat Thyroid Gland Maintained in Organ Culture (36595)

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(Introduced by E. M. Rivera)

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Using the organ culture technique, we have previously shown that TSH greatly improved ^{131}I utilization (1–3) but failed to influence the incorporation of ^{14}C-leucine into protein in embryonic rat thyroids (2, 3). On the other hand, insulin stimulated the incorporation of ^{14}C of various amino acids into protein (3, 4) in addition to stimulating ^{131}I incorporation into protein-bound iodotyrosines and thyroxine (3, 5). More recently (6, 7) we have studied the action of TSH both on the synthesis of various fractions of RNA as well as on the formation of thyroid hormones. TSH, added to the culture medium for 2 days, stimulated the synthesis of ribosomal RNA and apparently that of the messenger RNA as a whole. This increase in the transcription of RNA is paralleled by an increase in the metabolism of ^{131}I.

It was then of interest to study the action of insulin on the biosynthesis of various fractions of RNA as well as on the organic binding of iodine and on the incorporation of radioiodine into labeled soluble iodoproteins and, particularly, into thyroglobulin (19 S).

Materials and Methods. Thyroid glands were excised under sterile conditions from 21-day fetuses from CF (Wistar) rats and immediately placed in the culture media. The thyroid lobes were cultured by the method of Chen (8) and adapted to the culture of fetal rat thyroid by the method of Nataf *et al.*, as described previously (1, 5). Each culture dish contained 1 ml of medium in which a piece of dacron organdy was floated to support the two separate thyroid lobes (one gland). The basal medium used in these studies was Waymouth's medium (Gibco, 10×, glutamine and sodium bicarbonate-free, diluted in double distilled water, and the missing components added). The culture dishes were placed in a moisture-saturated air-tight chamber (Lwoff chamber equipped with a porthole) at a temperature of 37°. The atmosphere of the chamber consisted of a mixture of 92.5% O_2 and 7.5% CO_2, sterilized by Millipore filtration in order to maintain a pH of 7.3 (9).

TSH (Thytropar, Armour) was dissolved in a measured volume of a medium (5U/ml); an aliquot was added to the culture medium to obtain a concentration of 0.05 U/ml.

Insulin (Calbiochem) was dissolved in a minimum amount of 0.003 *N* HCl and diluted with the basal medium to provide a concentration of 5 μg of insulin/ml.

Ten to 20 μCi of carrier-free ^{125}I or 100 μCi of ^{32}P (H_3PO_4) were added after 2 days of culture; the culture dishes were then left in the incubator for another 24 hr.

^{125}I measurements. At the termination of the culture period, the fetal thyroid explants were rinsed with 0.14 *M* NaCl and homogenized in a solution (pH 8) containing 0.05 *M* tris (hydroxymethyl)aminomethane (Tris). Total ^{125}I uptake was determined by counting an aliquot of the homogenate; the remainder was digested for 6 hr with pronase at 37° under toluene and then chromatographed in collidine:3 *N* NH_4OH mixture. Radioautograms were prepared from the dried chromatograms. For quantitative estimation of ^{125}I in the various components, sections of the chromatograms corresponding to the radioautographic bands were cut out and counted in a well-type scintillation counter.

Incorporation of ^{125}I into soluble thyroidal proteins. At the end of the culture period, washed explants were homogenized in a pH 7.4 Tris–HCl buffer (0.05 *M* Tris–HCl in

0.14 *M* NaCl) and centrifuged at 4° for 1 hr at 4000*g*. The supernatant was then dialyzed overnight against the pH 7.4 buffer. An aliquot of the supernatant was applied to a 5–20% sucrose gradient in a Spinco model L2 centrifuge in a SW 65 rotor and spun at 125,000*g* for 5.5 hr.

RNA Preparation. The method previously described (10) for extracting different fractions of RNA was slightly modified. Washed thyroid explants were homogenized in 0.01 *M* Tris buffer (pH 7), 0.05 *M* NaCl, 0.002 *M* $MgCl_2$, in the presence of 500 μg of carrier RNA from mouse cells. The RNA referred to as "phenol-RNA" (RNA-p) was extracted by 4 successive phenol treatments. The interphase from the first phenol extraction was washed with the same buffer and precipitated with ethanol. The precipitate was resuspended in 2 ml of 0.015 *M* NaCl, 0.0015 *M* Na citrate buffer and incubated in the presence of Pronase and 1% sodium dodecyl sulfate. After 2 hr incubation at 37°, the RNA referred to as "interphase-RNA" (RNA-i) was finally extracted with phenol. The RNA-i was freed from DNA by treatment with DNAse (2 μg/ml, 30 min at 37°). After dialysis, the radioactivity incorporated into RNA-p and RNA-i was measured in a low-background flow counter. The different fractions were then separated by centrifugation in glycerol (11) or sucrose gradient.

Results. The present results (Table I) confirm our previous findings (1–3, 5) in showing that both insulin and TSH added to the culture medium greatly improved the functional activity of embryonic thyroid glands maintained in organ culture: after 2 days of culture, the 24 hr ^{125}I uptake as well as the incorporation of the isotope into protein-bound iodotyrosines and thyroxine were enhanced in the presence of either hormone. However, insulin was not so effective as TSH; indeed, the incorporation of radioactive iodine was much higher with TSH than with insulin. When added to the basal medium, TSH affected mostly the formation of labeled DIT and T_4. However, insulin, added to the basal medium, increased the proportion of MIT and DIT but not that of T_4, although the total amount of T_4 is almost double. As shown in Table I, when both insulin and TSH were present in the medium there was no additive effect.

Under the same experimental conditions, both insulin and TSH stimulate, in a similar fashion, the iodination of soluble proteins in each of the fractions recovered from the gland: insulin, however, is not so effective as TSH (Fig. 1). There does not seem to be a preferential increase in thyroglobulin 19 S fractions, since the sedimentation profiles (Fig. 1) show that the relative proportion of the different fractions separated by ultracentrifugation does not seem to be altered by the presence of insulin or TSH in the medium.

We have previously shown (6, 7) that TSH stimulated both the synthesis of ribosomal RNA and apparently that of "messenger" RNAs in general. Accordingly, we

TABLE I. Effects of TSH and of Insulin on 24 hr ^{125}I Uptake and Metabolism of Fetal Rat Thyroid Glands Maintained in Organ Culture.

^{125}I measurement	Culture in basal medium	Culture in basal medium + TSH	Culture in basal medium + insulin	Culture in basal medium + TSH + insulin
Uptake/thyroid (%)	2.76 ± 0.18[a]	11.40 ± 0.61	4.68 ± 0.17	11.44 ± 1.04
Distribution (%) on chromatograms				
MIT	39.57 ± 1.39	40.56 ± 1.93	53.77 ± 1.33	42.96 ± 0.69
DIT	18.16 ± 0.92	33.68 ± 1.92	27.0 ± 1.46	36.20 ± 0.51
$T_4 + T_3$	2.08 ± 0.12	6.96 ± 0.33	2.19 ± 0.23	6.32 ± 0.19
I^-	34.69 ± 2.58	13.01 ± 0.34	12.37 ± 0.89	9.06 ± 0.47

[a] Each value is the mean ± SE of the results of 9 separate experiments.

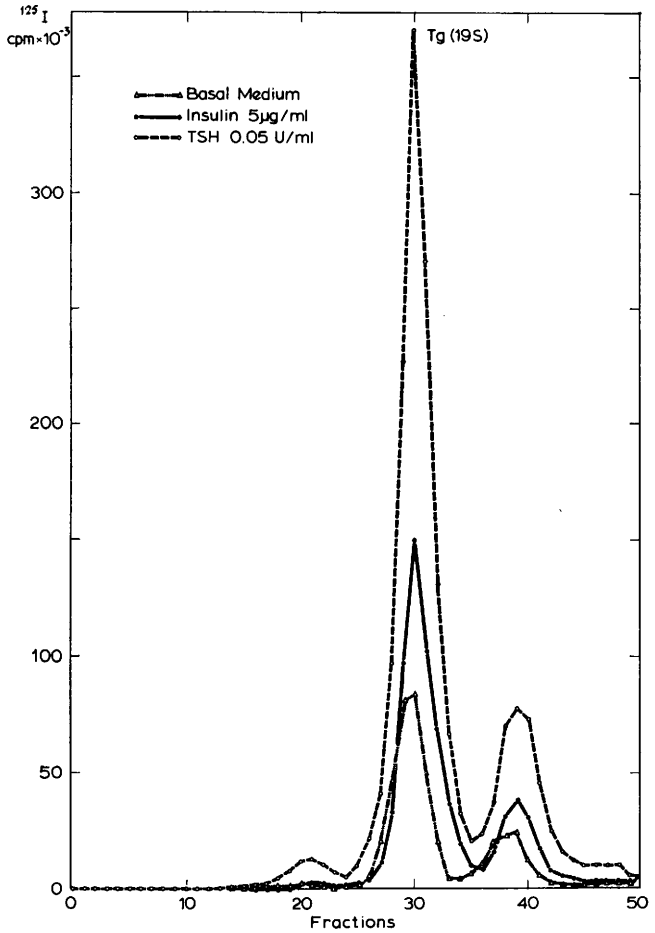

FIG. 1. Effects of TSH and of insulin on iodination of soluble proteins in fetal rat thyroid glands maintained in organ culture (24 hr incorporation of ^{125}I). The centrifugation was performed in 5–20% sucrose gradient for 5.5 hr (Spinco centrifuge, rotor SW 65, 41,000 rpm).

compared the effect of TSH and of insulin on the synthesis of various fractions of RNA. Although the effect of insulin and TSH varied in magnitude from one experiment to another, after 24 hr labeling, these two hormones consistently stimulated the incorporation of ^{32}P into RNA (Table II). The action of these hormones was about the same for RNA-p, which mostly represents the bulk of cytoplasmic RNA, and for RNA-i, which mostly consists of nuclear RNA (10). The stimulation induced by insulin was also confirmed, by comparing RNA extracted from purified nuclei (12) with RNA extracted from the cytoplasm. When insulin and TSH were added simultaneously to the culture medium, an additive effect of these two hormones was not consistently observed.

As in our previous experiments on fetal glands cultured in a basal medium (10), the sedimentation profiles of RNA-p and RNA-i obtained by ultracentrifugation in sucrose gradient (Figs. 2 and 3) show that RNA-p contains a negligible proportion of RNA heavier than the 28 S ribosomal RNA, whereas RNA-i is more polydisperse and contains a large amount of radioactive material sedimenting above 45 S and consisting of heavy-molecular weight DNA-like RNA (10). Figures 2 and 3 also demonstrate that for both RNA-p and RNA-i, the relative proportion of the different fractions of radioactive RNA

TABLE II. Stimulatory Effect of Insulin and TSH on the Incorporation of ^{32}P into Total or Fractionated RNA.[a]

Expt.	RNA	TSH	Insulin	TSH + insulin
1	Total RNA	—	187	268
2	Total RNA	216	—	276
3	RNA-p	159	—	252
	RNA-i	160	—	215
4	RNA-p	195	273	317
	RNA-i	184	270	315
5	RNA-p	175	210	242
	RNA-i	256	324	326
6	RNA-p	217	229	355
	RNA-i	225	255	316
7	Cytoplasmic RNA	—	231	—
	Nuclear RNA	—	181	—

[a] Each value represents the total radioactivity (cpm) incorporated into RNA extracted from hormone-treated glands/total radioactivity in RNA from untreated glands × 100.

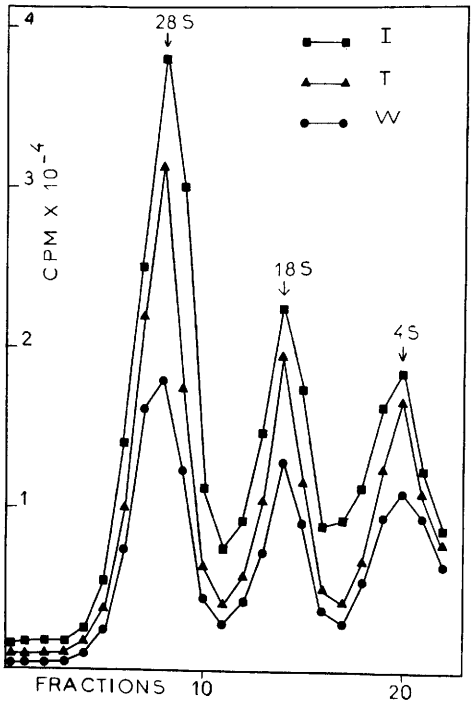

FIG. 2. Labeled phenol-RNA centrifuged in 5–20% sucrose gradient, 0.01 *M* Tris, 0.05 *M* NaCl; 0.001 *M* EDTA at 24,000 rpm for 16 hr in the SW25 of Spinco ultracentrifuge. (●—) Culture in basal medium (W); (▲—) culture in basal medium + 0.05 U TSH/ml (T); (■—) culture in basal medium + 5 μg insulin/ml (I); (arrows) 28S, 18S, 4S: determined by optical density (260 nm) of carrier RNA.

does not seem to be modified by TSH or insulin; in other words, both hormones increased similarly the ^{32}P incorporation into ribosomal RNA, transfer RNA and DNA-like nuclear RNA.

Discussion. The present results confirm our previous observations (3) on the stimulatory effect of TSH and insulin on iodine metabolism, TSH being much more active than insulin; however, when both hormones were present in the medium, there was no additive effect on iodine metabolism in contrast to what we observed previously under different conditions (3): (a) The duration of the culture was previously 4 days in the medium 199 instead of 3 days in Waymouth's medium in this study. In both cases, histological examinations were made to control the viability of the tissue in the basal medium used, no sign of cellular degeneration was observed. (b) The insulin previously used was amorphous insulin (Eli Lilly) instead of crystallin insulin (Calbiochem). (c) Finally, the animals used previously were from Long-Evans strain instead of Wistar in the present study. It is possible that one or all these differences in the experimental conditions might explain the discrepancy between the results obtained.

The present results show also that the iodination of thyroglobulin (19 S) as well as that of the other soluble iodoproteins was

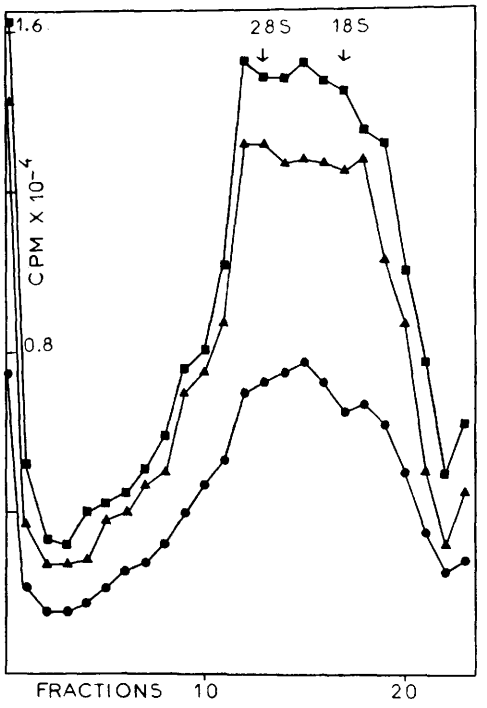

FIG. 3. Labeled interphase-RNA centrifuged in 5–20% sucrose gradient, 0.01 *M* Tris, 0.05 *M* NaCl, 0.001 *M* EDTA at 17,500 rpm for 16 hr in the SW25 of Spinco ultracentrifuge. (●—) Culture in basal medium (W); (▲—) culture in basal medium + 0.05 U TSH/ml (T); (■—) culture in basal medium + 5 μg insulin/ml (I); (arrows) 28S, 18S: determined by optical density (260 nm) of carrier RNA.

increased in the presence of either hormone; however, TSH was much more active than insulin. TSH stimulates both the synthesis of ribosomal RNAs, of DNA-like nuclear RNAs and, as previously described (6), that of rapidly labeled RNAs in general.

The mode of action of TSH on biosynthesis of RNA is not yet clear. According to various authors (13–18) one site at which TSH may affect nucleic acid synthesis is the formation of nucleotides which is mediated by stimulation of glucose oxidation via hexose monophosphate pathway, thereby increasing the supply of available glucose. For Hall and Tubmen (16) and Lindsay, Cash and Hill (18), the *in vitro* effect of TSH on RNA synthesis cannot be blocked by puromycin and therefore does not depend on the formation of new protein. The results of others (19–22) indicate another site at which TSH can affect the synthesis of nucleic acids, the polymerization of nucleotides. For Begg and Munro (19, 20), the stimulatory effect of TSH on RNA synthesis, which is blocked by puromycin, requires the preliminary formation of a specific nuclear protein. Moreover, Adiga, Murthy and McKenzie (23) reported that TSH increased the activity of RNA polymerase in porcine thyroid slices.

From our results as well as from those cited above, it is not yet possible to conclude that TSH acts by inducing the transcription of specific messenger RNAs, although this hypothesis cannot be excluded.

The mode of action of insulin may well depend on its ability to promote the entry of glucose or amino acids or both into tissues (24, 25). In effect, insulin has been shown to increase glucose uptake (26) and incorporation of ^{14}C-amino acids into protein in various organs maintained in organ culture (27–31) including fetal rat thyroid glands (3, 4). The various actions of insulin described in this study might be due in part to its stimulation of protein synthesis, although the influence of some other mechanism, such as its effect on glucose metabolism cannot be excluded. However, since Field *et al.* (32) and Merlevede, Weaver and Landau (33) do not find any effect of insulin on the oxidation of glucose by adult thyroid slices, it might be, then, rather unlikely that the various effects of insulin cited here are mediated only via the influence of this hormone on glucose metabolism. The results of Singh and Chaikoff (34) suggested that the influence of insulin on protein synthesis is not entirely dependent on the stimulation of messenger RNA formation.

The present study shows that insulin, as TSH, increases quite similarly the 24 hr ^{32}P incorporation into ribosomal RNA, transfer RNA and DNA-like RNA. It is conceivable that the various effects of both hormones are primarily due to their influence on the metabolism of ribonucleic acid.

Summary. In organ culture of fetal rat thyroid glands, both insulin and TSH en-

hance significantly the 24 hr incorporation of ^{125}I into protein-bound iodotyrosines and iodothyronines; however, TSH is much more active than insulin. Under the same experimental conditions, although insulin is not so effective as TSH, these two hormones stimulate, in a similar fashion, the iodination of soluble proteins. Both TSH and insulin enhance also the biosynthesis of ribosomal RNAs and of nuclear DNA-like RNAs.

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