Thyrotropin-Like Activity of Thyroid RNA *in Vitro* (37075)

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(Introduced by P. G. Dayton)
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Exogenous RNA can enter the recipient cell as an undegraded macromolecule (1–4), and control protein and enzyme synthesis (5–8), as well as the development of the embryo (9–12). Further, the target tissues of hormone-deprived animals have been shown to respond to the application of RNA from specific tissues of animals stimulated with sex hormones (13–17). The purpose of the present study was to learn whether RNA from TSH-treated thyroid has the ability to stimulate iodine metabolism of TSH-deprived thyroid tissue to the same extent as TSH itself.

Material and Method. Male albino guinea pigs, weighing 400–500 g, which were fed chicken feed, supplemented with fresh vegetables and water *ad lib.*, were used for RNA preparations. TSH (thyrotropin, Schwarz/Mann, Orangeburg, NY, 0.25 IU in 0.25 ml of normal saline) was injected subcutaneously 12 and 24 hr before sacrificing the animals.

Other guinea pigs from the same strain, weighing 250–350 g were used for organ culture. These animals were injected daily with T_3 (Sigma Chemical Co., St. Louis, MO, 20 μg in 0.1 ml im) for 1 wk prior to sacrificing the animals.

Thyroid and liver RNA were prepared by a modification of the method of Kirby (18). The thyroid glands of 12 TSH-treated guinea pigs were pooled for each experiment. The animals were sacrificed by a blow on the head, the glands were removed immediately and placed in 10 ml of cold (4°) (pH 5.0) 0.01 *M* acetic acid–sodium acetate buffer containing bentonite (5 mg/ml BDH), sodium dodecyl sulfate (5 mg/ml) and polyvinyl sulfate (5 μg/ml, BDH). The glands were homogenized at 4° and the volume was brought to 15 ml with the buffer. Then, 15 ml of 90% phenol was added with stirring and the mixture was incubated at 50° for 4 min. After cooling to 4° and centrifugation (1200*g* at 4° for 40 min), the supernatant was passed through an IRA-400 column (Amberlite, BDH, 0.2 cm diameter 10 cm length) to remove iodine (19). The eluate was collected and 2.5 vol of absolute ethanol (4°) was added with stirring. The precipitated RNA was collected by centrifugation (10,300*g* at 0° for 30 min). The supernatant was discarded and the precipitate was dried *in vacuo* at 4°. It was then dissolved in 5 ml 0.01 *M* Tris buffer, pH 7.4. To the RNA solution, 10 μg/ml of DNase was added (Sigma) as described by Fujii and Villee (20). After allowing the mixture to stand for 10 min at room temperature, the RNA was precipitated at 4° with cold absolute alcohol. RNA was removed by centrifugation as before. Liver RNA was prepared by the same procedure. The nucleotides were measured spectrophotometrically at 230, 260 and 280 nm. Denatured thyroid RNA was prepared by hydrolysis (1 hr) of the RNA with RNase (Sigma, 20 μg/mg RNA) at room temperature.

The thyroid glands of the T_3-treated animals were removed under sterile conditions. Each pair of glands was placed on a sterilized glass pad (Sartorium membrane filter GMBIT SH13400). Then, four pads were placed on a plastic petri dish (60 × 15 mm, Falcon Plastic Division, Bioquest). Medium was added to cover the glass pad, exposing the gland to gases. The medium was prepared from 10% (v/v) newborn calf serum in Eagle's essential medium (Hyland Division, Travenol Labs, Inc.); the pH was adjusted to 7.4 with 0.2 *N* sodium bicarbonate. The petri dishes were placed in a shaking machine

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with 36 up and down excursions/min, which in turn was placed in a CO_2 incubator (equipped with CO_2 mixer, Lab Line, Inc.) at 37° with 5% CO_2 in air. After 12-hr preincubation, each pair of glands was transferred into a plastic petri dish (35 × 10 mm, Falcon). Fresh medium containing 0.2 μCi of carrier-free ^{131}I/petri dish was added. TSH, thyroid RNA, or liver RNA were then added. After an additional 24-hr incubation, iodine metabolism was determined as follows: the thyroid glands were washed with (pH 8.0) Tris buffer (NaCl, 0.15 *M*; Tris, 0.04 *M*, $CaCl_2$, 0.014 *M*, methimazole, 0.002 *M*). After blotting, and weighing, the glands were digested with 1 ml of 0.5% proteolytic enzyme (Pronase, Calbiochem) in Tris buffer at 37° for 8 hr. The radioactivity of a standard solution (0.2 μCi ^{131}I in 1 ml Eagle's medium) and digested thyroid glands were measured with a well scintillation counter, and the ratio was determined.

The iodinated amino acids in the enzyme-digested thyroid tissue were separated with paper chromatography (descending system: *n*-butanol:water:glacial acetic acid, 78:17:5). Radioactivity was detected with a paper strip radiochromatograph scanning system (Nuclear Chicago). The release of ^{131}I-T_4 and ^{131}I-T_3 into the medium was detected by chromatography on IRA-400 resin (19) as protein bound (PB) ^{131}I.

Biological activity of thyroid RNA was measured as follows:

Experiment I. Ten pairs of preincubated thyroid glands were divided into two groups (1A and 1B). The usual medium containing 2 μCi ^{131}I was used in group 1A for control purposes. In group 1B, 40 mIU of TSH was added. After 24-hr incubation, four pairs of glands from each group were used for determination of iodine metabolism and one pair for histological examination. The above experiments were repeated two more times.

Experiment II. Three groups of experiments involving the effect of thyroid RNA on iodine metabolism *in vitro* were carried out (IIA, IIB and IIC). Group IIA was used as a control. For group IIB, 200 μg of thyroid RNA was added to the medium/dish while in group IIC, 200 μg of RNase-treated thyroid RNA was added. The determinations were the same as in Expt. I.

Experiment III. Determination of the specificity of biological activity of thyroid RNA was investigated as follows:

The experiment was carried out in three groups (IIIA, IIIB and IIIC). Group IIIA served as a control, IIIB was treated exactly like IIB, and in group IIIC, 200 μg of liver RNA was added/dish. The determinations were the same as in Expt. I.

For histological examination one pair of thyroid glands from each group was used. If necrotic changes were observed, the data from that particular experimental group was discarded.

Results. In tissue culture experiments the uptake of ^{131}I was significantly stimulated by TSH and thyroid RNA but not by liver RNA; it was even decreased by RNase-treated thyroid RNA (Table I).

Release of PB ^{131}I (which was used as an indication of hormonal release from thyroid gland to the medium) was again significantly higher in both the TSH- and the thyroid RNA groups than that of the control groups. There was no significant difference between the control group and liver RNA or RNase-treated groups (Table I).

The incorporation of ^{131}I into the amino acids of the thyroid tissue is shown in Table II. The ratio of radioactivity of T_3 and T_4 was increased by the stimulation both by TSH and thyroid RNA. This increase could not be found in the thyroid tissue treated with liver RNA or denatured thyroid RNA.

Discussion. It is known that TSH stimulates iodine metabolism of thyroid gland *in vivo* (21). Our results *in vitro* show that the 24-hr uptake of ^{131}I by thyroid gland of TSH-deprived guinea pigs was stimulated by the addition of TSH to the medium. The incorporation of ^{131}I into T_3 and T_4 in the thyroid glands and release of PB ^{131}I to the medium also were higher in the TSH group than that in controls. These findings demonstrate conclusively that iodine metabolism of thyroid gland can be stimulated by TSH *in vitro*. Similarly, the thyroid gland of TSH-deprived guinea pigs responded with increased iodine metabolism when an RNA preparation from

TABLE I. 24-Hour Uptake of ^{131}I and PB ^{131}I.[a]

Group	No. of guinea pigs	24-hr ^{131}I uptake/mg of thyroid tissue (%)[b]		24 hr PB ^{131}I/total added radioactivity (%)[c]	
		Mean ± SD	p[d]	Mean ± SD	p[d]
Control	36	0.32 ± 0.05		0.83 ± 0.11	
TSH	36	0.54 ± 0.10	<.001	1.43 ± 0.20	<.001
T-RNA[e]	24	0.40 ± 0.05	<.05	1.63 ± 0.24	<.001
RNase[f]	12	0.23 ± 0.01	<.05	0.90 ± 0.11	>.1
L-RNA[e]	12	0.29 ± 0.01	>.5	0.69 ± 0.11	>.1

[a] The weight of the thyroid glands of T[3]-treated guinea pigs was 23.7 ± 4.3 SD mg (N = 144). This is significantly lower than in the controls (35.7 ± 4.6 mg; N = 12). The total amount of thyroid RNA extracted from 12 pairs of TSH-treated thyroid glands (500–600 mg) was about 5–6 mg.

[b] Expressed as [(cpm uptake/mg gland)/cpm added] × 100.

[c] Expressed as (cpm of PB ^{131}I/cpm added) × 100.

[d] Significance between control groups and others.

[e] T = thyroid, L = liver.

[f] RNase-treated.

thyroid gland of TSH-treated guinea pigs was added to the medium. The TSH-like effect of thyroid RNA of TSH-treated guinea pigs confirms the findings that the uterus or seminal vesicles of immature or hormone-deprived animals respond to the RNA from specific tissues of animals stimulated with sex hormones (13–17).

Thyroid RNA denatured with RNase had no effect on I^- metabolism. This shows that the TSH-like effect of thyroid RNA was not due to contamination of RNA with TSH. Furthermore, the iodine metabolism of the thyroid gland of TSH-deprived guinea pigs was not stimulated by an RNA preparation from the liver of TSH-treated guinea pigs. Fencl and Villee (15) found that intrauterine application of hepatic RNA increased protein synthesis, just as uterine RNA. In contrast, evidence for the organ specificity of RNA was presented by Sanyal and Niu (22). Fujii and Villee (17) reported that RNA preparations from organs other than the seminal vesicle (testosterone-treated immature rats) did not produce a significant increase in the weight of the seminal vesicle. Our findings indicate that under our experimental conditions the effect of RNA is organ specific.

Summary. The biological effect of RNA preparations from the thyroid glands of TSH-stimulated guinea pigs was studied. In particular, iodine metabolism of the thyroid gland was stimulated by both TSH and thyroid RNA *in vitro* but not by liver RNA or RNase-treated thyroid RNA. The results indicate that thyroid RNA mediates the TSH-

TABLE II. The ^{131}I Distribution in the Iodoamino Acids in the Thyroid Glands.

Group	No. of guinea pigs	Percentage[a]				
		^{131}I	MIT	DIT	T_3 and T_4	p[b]
Control	36	12.58 ± 5.78	43.23 ± 9.58	39.05 ± 5.28	5.15 ± 2.15	
TSH	36	16.34 ± 7.24	28.77 ± 2.96	39.09 ± 4.31	15.87 ± 6.33	<.005
Thyroid RNA	24	21.31 ± 5.99	37.73 ± 14.22	26.58 ± 9.65	14.40 ± 2.99	<.005
RNase[c]	12	22.64 ± 6.47	33.53 ± 7.23	37.51 ± 11.37	6.32 ± 1.58	>.2
Liver RNA	12	18.69 ± 4.51	45.10 ± 9.16	29.90 ± 13.21	6.32 ± 1.53	>.2

[a] Percentage of radioactivity in particular area; mean ± SD. MIT = monoiodotyrosine, DIT = diiodotyrosine.

[b] p value given for (T_3 and T_4).

[c] Treated with RNase.

stimulated iodine metabolism in the thyroid. This TSH-like effect of thyroid RNA is organ-specific and not due to contamination of RNA with TSH.

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