Short Communications

Stimulation by Thyrotrophin of [^3H]Uridine Incorporation in the Polysomes of Thyroid Slices

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(Received 30 May 1967)

Thyrotrophin has two different types of effects on the thyroid: it enhances, almost immediately, the synthesis and the secretion of thyroid hormone, and it promotes, with some delay, cellular and tissular growth. It is probable that the latter effects, but not the former, require an intact RNA synthesis (Schneider & Goldberg, 1965; Tong, 1965; Taurog & Thio, 1966). Thyrotrophin *in vitro* stimulates the incorporation of phosphate, formate, glycine and adenine into the RNA of thyroid slices (Hall, 1963; Hall & Tubmen, 1965; Begg & Munro, 1965; Shimada & Yasumasu, 1966). It is not known into which RNA this increased incorporation takes place. The hypothesis has been proposed that thyrotrophin acts by stimulating the synthesis of messenger RNA in thyroid nuclei (Begg & Munro, 1965). A first test of this hypothesis is to ascertain whether thyrotrophin does enhance the incorporation of precursors into thyroid messenger RNA. We have performed this test with the polysomes of stable sheep thyroid-slice preparations (Lecocq & Dumont, 1966).

Methods. The techniques of incubation of sheep thyroid slices and the methods used for the statistical evaluation of the data have been previously described (Dumont, 1964; Lecocq & Dumont, 1966). Approx. 1g. of slices was incubated in 13ml. of Krebs-Ringer bicarbonate buffer enriched with culture medium 199 and calf serum (10%, v/v). After a 2hr. preincubation $50\mu\text{C}$ of [^3H]uridine (The Radiochemical Centre, Amersham, Bucks.) and, when required, 2 i.u. of thyrotrophin (Thytropar; Armour, Kankakee, Ill., U.S.A.) were added to the medium and incubation was continued for another 4hr. The ribosomes of the slices were prepared and analysed by density-gradient centrifugation (Lecocq & Dumont, 1966). Typical ribosome patterns are shown in Fig. 1. From these patterns we evaluated $P/(P+M)$ ratios (where P and M are the E_{260} values corresponding to polysomes and monosomes respectively) and the specific activity of the polysomes in disintegrations/min./cm.² of the polysomal peak area.

Ribosomes were dissociated into 30s and 50s

particles by passage through a CM-cellulose column (Marbaix & Burny, 1964). The particles were separated by centrifugation on a 5–20% (w/v) sucrose gradient in 50mm-phosphate buffer, pH 7.4. The centrifugation was carried out for 3.5hr. at 39000rev./min. in a Spinco centrifuge (SW39 rotor). The RNA of the ribosomes was freed by a treatment with sodium dodecyl sulphate (Kurland, 1960; Staehelin, Wettstein, Oura & Noll, 1964) and separated by centrifugation on a 5–20% (w/v) sucrose gradient in 5mm-tris-HCl buffer, pH 7.4. The centrifugation was carried out for 6hr. at 39000rev./min. in a Spinco centrifuge (SW39 rotor).

Purified thyrotrophin (6 i.u./mg.) was a gift from Dr P. G. Condliffe.

Results and discussion. In control slices as well as in thyrotrophin-stimulated slices, [^3H]uridine was mainly incorporated into the polysomal fraction of the ribosomes (Figs. 1a and 1c). Thyrotrophin stimulated this incorporation from 196 to 572 disintegrations/min./cm.². Similar results were obtained in eight experiments. Purified thyrotrophin had a similar effect. Actinomycin D inhibited the incorporation of [^3H]uridine into the polysomes of control (Lecocq & Dumont, 1966) and stimulated slices (Fig. 1b). This incorporation therefore does not correspond to the turnover of the terminal nucleotides of transfer RNA. After a slight treatment of the ribosomes with ribonuclease (0.01 $\mu\text{g.}/\text{ml.}$ at 37° for 5min.) most of the ribosomes were found in the monosome peak, whereas most of the radioactivity had shifted to the soluble fraction on top of the gradient (Fig. 1d). The RNA into which [^3H]uridine had been incorporated was therefore not ribosomal RNA.

After dissociation of the ribosomes on CM-cellulose, the radioactivity was polydispersed, overlapping on both sides the areas of the 30s and 50s peaks. Treatment of the ribosomes with ribonuclease before the dissociation caused a shift of the radioactivity to the top of the gradient well apart from the 30s and 50s peaks. After dissociation of the ribosomes with sodium dodecyl sulphate, the radioactivity was polydispersed, extending on both

Fig. 1. Ribosome patterns of sheep thyroid slices incubated in the presence of [^3H]uridine. (a) Control slices; (b) slices incubated in the presence of thyrotrophin and actinomycin D; (c) thyrotrophin-stimulated slices; (d) thyrotrophin-stimulated slices, the ribosomes having been slightly hydrolysed with ribonuclease. —, E_{260} ; $\times$ — $\times$, radioactivity.

sides beyond the limits of the 16s and 28s peaks. Little radioactivity was found in the 4–5s region. Treatment of the ribosomes with ribonuclease before the dissociation caused a shift of the radioactivity to the top of the gradient away from the 16s and 28s peaks. Similar results were obtained with the polysomes of control and stimulated slices. These results suggest that in control as in stimulated slices the main incorporation of [^3H]uridine into polysomes does not take place at the level of transfer or ribosomal RNA but at the level of messenger RNA.

In all the experiments performed, the $P/(P+M)$ ratio was increased in thyrotrophin-stimulated slices. In eight experiments the logarithmic mean of the ratio was 0.68 in control and 0.76 in stimulated slices ($P < 0.001$). These findings indicate a shift in the ribosome pattern from the monosomes to the polysomes. They suggest that thyrotrophin induces a net synthesis of messenger RNA in sheep thyroid slices *in vitro*. They support the hypothesis that some effects of thyrotrophin on the thyroid,

presumably delayed effects, are secondary to an activation of messenger RNA synthesis.

This work was carried out under Contract Euratom–University of Pisa–University of Brussels no. 026-63-4 BIAC, and under Contract F.R.F.C. 22 of the Fonds de la Recherche Fondamentale Collective.

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