EFFECT OF THYROTROPHIN RELEASING HORMONE ON SECRETION OF THYROTHROPHIC HORMONE AND PROLACTIN IN A MONOLAYER CULTURE OF RAT ADENOHYPOPHYSIS

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Cells of the adenophypophysis in a primary 5-8-day monolayer culture react to addition of thyrotrophin releasing hormone (TRH) by rapid dose-dependent liberation of thyrotrophic hormone (TSH) and prolactin into the culture medium. This effect is independent of the content of serum in the nutrient medium. The thyroid hormone thyroxin blocks the stimulating action of TRH on the secretion of TSH, but not of prolactin. The blocking effect of thyroxin is evidently manifested not on cell membranes, but in the cytoplasm distally to the effect of cyclic AMP, along the pathway of transmission of the hormonal signal.

KEY WORDS: culture of adenophypophysis; thyrotrophin; prolactin; thyrotrophin releasing hormone; thyroxin.

The development of methods of long-term culture of adenohypophyseal cells in vitro in the last 5 years has opened a new stage in the investigation of the mechanism of secretion of the trophic hormones and of hypothalamo-hypophyseal regulation. Fundamental studies have been carried out on cell cultures to determine the biological activity of hypothalamic peptides and their synthetic analogs controlling the secretion and synthesis of adenohypophyseal hormones [5, 7, 11, 14]. Work is currently in progress to study the intracellular mechanisms of action of releasing hormones, their mutual effects, and the role of the peripheral glands of internal secretion and of other factors in the functioning of so important an endocrine organ as the pituitary gland.

The object of the present investigation was to study the characteristics of secretion of thyrotrophic hormone (TSH) and prolactin by a culture of adenohypophyseal cells, depending on the conditions of culture and the content of thyrotrophin releasing hormone (TRH) and thyroid hormone (thyroxin; T_4) in the medium.

EXPERIMENTAL METHOD

The adenohypophyses were isolated from male Wistar rats weighing 150-200 g after decapitation. With the aid of 0.25% trypsin and mechanical separation of the tissue a cell suspension was prepared by the method described previously [9]. Cells were seeded on plastic petri dishes (diameter 1 cm, Falcon Plastics, USA), arranged with 20 dishes on one tray and with $8 \cdot 10^5$ cells per dish. The cells were cultured for the first 48 h in medium No. 199 with the addition of 20% embryonic calf serum in an atmosphere of air containing 5% CO_2. Later the quantity of serum was reduced to 10%. The medium was changed after 3 days. Experiments were carried out on 5-8-day cultures. Before the beginning of the experiment the medium was removed from the dishes, and the dishes were washed twice with fresh medium. TRH (Hoechst, West Germany) was added in doses of 1-100 ng/ml to medium containing or not containing serum, depending on the experimental conditions. Sodium dibutyryl cyclic AMP (dbc-AMP) was added in a dose of $5 \cdot 10^{-3}$M. Preincubation with thyroxin (Reanal, Hungary) in a dose of 10 μg/ml was carried out for 1 h before addition of TRH or dbc-AMP, after which it was removed from the medium. The concentrations of TSH and prolactin in the medium were determined by a radioimmunological method [1].

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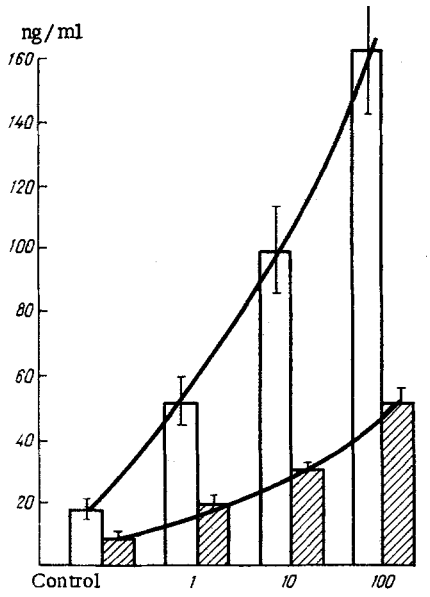

Fig. 1

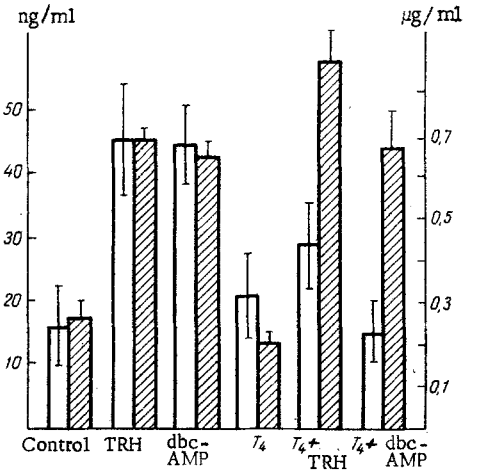

Fig. 2

Fig. 1. Effect of different doses of TRH on liberation of TSH and prolactin by adenohypophyseal cells in culture 15 min after addition. Unshaded columns) TSH; shaded columns) prolactin. Abscissa, doses of TRH (in ng/ml); ordinate, hormone concentration (in ng/ml medium).

Fig. 2. Effect of thyroxin (T_4) on basal secretion of TSH and prolactin and their secretion stimulated by TRH and dbc-AMP. Preincubation with T_4 (10 μg/ml) for 1 h, followed by incubation with TRH (100 ng/ml) or dbc-AMP (5×10^{-3}M) for 3 h. Unshaded columns denote TSH; shaded columns prolactin. Ordinate: left, concentration of TSH (in ng/ml medium); right, prolactin concentration (in μg/ml medium).

TABLE 1. Action of TRH (100 ng/ml) on Content of TSH and Prolactin in Medium under Different Conditions of Culture of Adenohypophyseal Cells (M ± m)

Conditions of culture	Composition of nutrient medium	Duration of incubation with TRH, h	Concentration of TSH in medium, ng/ml	Prolactin concentration in medium, ng/ml
Control	Medium with serum	24	157,3±37,3	416,7±4,4
TRH		24	247,3±63,7	374,3±73,7
			$P>0,05$	$P>0,05$
Control	Medium without serum	24	17,0±5,9	
TRH		24	63,3±16,0	
			$P>0,05$	
		1/2	50,5±5,6	
			$P<0,05$	

EXPERIMENTAL RESULTS

Within 15 min after its addition TRH induced a dose-dependent increase in the liberation of prolactin and TSH by the adenohypophyseal cells into the culture medium (Fig. 1). The minimal effective dose of TRH was 1 ng/ml. In a dose of 100 ng/ml, TRH led to a five- and eightfold increase, respectively, in the levels of prolactin and TSH in the medium. The magnitude of the secretory response of the adenohypophyseal cells could vary from one experiment to another, evidently because of variation in the number of hormone-secreting cells in each particular culture [3]. However, the dose dependence was constantly close to linear.

Prolonged incubation of the cells with a single addition of TRH in a dose of 100 ng/ml did not lead to any marked increase in the content of TSH or prolactin in the medium compared with the control (Table 1), although TRH is known to stimulate not only the release, but also the synthesis of these hormones [4, 8]. TRH was evidently destroyed rapidly in the medium, and repeated additions of the hormone were necessary to obtain an effect [13].

Removal of the serum from the nutrient medium sharply delayed the secretion of TSH in the control and experimental groups (Table 1). The TSH concentration in the later stages after addition of TRH was higher than in the control group. A similar effect was produced by incubating the cells for a short time with TRH in a dose of 100 ng/ml after they had been kept for 24 h in medium without serum.

Cells of the adenohypophysis in culture thus react to TRH by the liberation of TSH regardless of whether serum is present in the medium or not.

The results can be summarized by the statement that a single dose of TRH caused the rapid release of TSH and prolactin into the culture medium. Later, however, this effect was canceled out by constant secretion of hormones, independent of TRH, into the medium, so that after 24 h the concentrations of TSH and prolactin in the two groups did not differ significantly. After removal of the serum from the medium the basal secretion of TSH was reduced to such a degree that the hormone liberated into the medium immediately after addition of TRH brought about a substantial difference in concentrations compared with the control.

The inhibitory effect of T_4 on stimulation of TSH and prolactin secretion induced by TRH or dbc-AMP was studied (Fig. 2). Both dbc-AMP and TRH stimulated the liberation of both hormones equally into the medium. These data are in good agreement with the results obtained by other workers and they indicate that the mechanism of action of TRH is mediated through cyclic AMP [6, 10]. The insufficiently high basal level of TSH secretion and the comparatively weak response to TRH were evidently due to the low concentration of thyrotrophs in the particular culture.

Preincubation of the cells with T_4 under these conditions did not affect the basal secretion of TSH and prolactin. Meanwhile T_4 reduced the stimulating effect of TRH and completely abolished the action of dbc-AMP on TSH secretion but did not affect the liberation of prolactin. These results indicate that thyroid hormones inhibit the stimulating effect of TRH not at the level of hormone–receptor interaction, but along the pathway of intracellular transmission of the hormonal signal. The view is held that thyroid hormones can promote synthesis de novo of a substance of protein nature which inhibits the effect of TRH on TSH secretion [12]. In this respect the data showing that thyroid hormones increase the activity of phosphodiesterase, which causes degradation of cyclic AMP [2], are interesting.

It is difficult at present to explain why the same dose of T_4 abolished the effect of dbc-AMP completely but that of TRH only partially. Some workers consider [4] that TRH acts not only through cyclic AMP, but also by some other way on the target cell. On the other hand, it can be tentatively suggested that the analog of the cyclic nucleotide is not absolutely identical in its action with cyclic AMP. Finally, the high specificity of action of T_4 by the feedback principle must be emphasized once again, for the secretion of TSH but not of prolactin falls within its sphere of influence.

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