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EFFECT OF THYROTROPIN RELEASING HORMONE AND THYROXINE ON CYTOCHROME OXIDASE ACTIVITY IN THE RAT ADENOHYPOPHYSIS

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Thyrotropin releasing hormone (TRH), in a dose of 0.01 and 1.0 μg/ml, sharply increased cytochrome oxidase activity in the adenohypophysis of rats fed for 6 weeks with methylthiouracil. This effect of TRH on enzyme activity was blocked by thyroxine (T_4), if added to the incubation medium in a concentration of 20 μg/ml. Actinomycin D (20 μg/ml) prevented the blocking of cytochrome oxidase by T_4. TRH in a concentration of 0.01 μg/ml and T_4, in a dose of 2.0 μg/ml, caused no change in cytochrome oxidase activity in the adenohypophysis of intact and partially thyroidectomized rats.

KEY WORDS: thyrotropin releasing hormone; thyroxine; cytochrome oxidase; adenohypophysis.

Little information is available on the effect of releasing hormones on metabolic processes accompanying the secretion of the pituitary tropic hormones. The stimulating effect of thyrotropin releasing horming (TRH) on the formation of $^{14}CO_2$ from [^{14}C]glucose by pig [7, 11] and rat [8] pituitary glands and the blocking of this effect by thyroxine (T_4) have recently been described [7, 11].

Since the intensity of tissue respiration is known to depend on the state of the cytochrome system, including cytochrome oxidase activity [4], it was decided to study cytochrome oxidase activity in rat pituitary glands under the influence of TRH and T_4.

EXPERIMENTAL METHOD

Intact rats and rats in which the sensitivity of the thyrotrophs of the adenohypophysis was increased by partial thyroidectomy or by feeding the animals daily for 6 days (5 mg per animal) with methylthiouracil were used. The adenohypophysis of the rats, after decapitation, was divided into halves (one half was the control, the other experimental). Five or six halves of adenophypophyses were washed, weighed together, and placed in Erlenmeyer flasks with 2 ml of Hanks' medium, aerated with a mixture of 95% O_2 + 5% CO_2 to complete saturation. The samples were then placed in an apparatus of Warburg type for preincubation. After 1 h, TRH (from Hochst, West Germany), T_4, or actinomycin D (from Reomal, Hungary) in 0.5 ml Hanks' medium was added to the experimental samples and 0.5 ml of Hanks' medium only to the control. Cytochrome oxidase activity, de-

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TABLE 1. Effect of TRH and T_4 on Cytochrome Oxidase Activity in Adenohypophyses of Intact and Partially Thyroidectomized Rats (M ± m)

Animals	Conditions of incubation	Number of determinations	Cytochrome oxidase activity		P
			mg tetrazolium/100 mg tissue	%	
Intact	Control	5	0,23±0,02	100	
	TRH, 0.01 μg/ml (10 min)	5	0,29±0,02	125	>0,05
	TRH, 1 μg/ml (10 min)	5	0,41±0,02	178	<0,05
	Control	6	0,37±0,08	100	
	T_4, 20 μg/ml (120 min)	6	0,43±0,01	116	>0,05
Partially thyroidectomized	Control	6	0,23±0,05	100	
	TRH, 0.01 μg/ml (10 min)	6	0,29±0,04	125	>0,05
	Control	6	0,27±0,05	100	
	T_4, 20 μg/ml (120 min)	6	0,30±0,02	111	>0,05

TABLE 2. Effect of TRH and T_4 on Cytochrome Oxidase Activity in Adenohypophyses of Rats after Feeding with Methylthiouracil for 6 Weeks (M ± m)

Animals	No. of determinations	Cytochrome oxidase activity		P
		mg tetrazolium/100 mg tissue	%	
Control	6	0,55±0,09	100	
TRH, 0.01 μg/ml (10 min)	6	0,93±0,16	169	<0,05
Control	6	0,59±0,06	100	
TRH, 1.0 μg/ml (10 min)	6	1,181±0,05	200	<0,001
Control	6	0,57±0,05	100	
T_4, 2.0 μg/ml (120 min)	6	0,54±0,05	94	
Control	6	0,56±0,05	100	
T_4, 20.0 μg/ml (120 min)	6	0,30±0,02	54	<0,05
Control	6	0,54±0,02	100	
T_4, 20 μg/ml (60 min) + TRH, 1 μg/ml	6	0,54±0,01	100	
T_4, 20 μg/ml (120 min) + TRH, 1 μg/ml	6	0,24±0,07	54	<0,05
Actinomycin D, 20 μg/ml (120 min) + T_4, 20 μg/ml (60 min)	7	0,50±0,06	92	
Control	6	0,57±0,07	100	
TRH, 1.0 μg/ml (10 min)	6	0,87±0,04	152	<0,05
Actinomycin D (120 min)	6	0,54±0,05	95	
Actinomycin D (120 min) + TRH, 1.0 μg/ml (10 min)	6	0,76±0,02	133	<0,05
Actinomycin D (120 min) + T_4 (60 min) + TRH (10 min)	6	0,86±0,06	152	<0,05

TABLE 3. Effect of TRH and T_4 on Concentration of Immunoreactive TTH (in milliunits/ml) in Rat Blood Plasma (M ± m)

Control	TTH concentration			
	30 min after injection of TRH (0.01 μg/ml)	2 h after injection of T_4 (20 μg/ml)	30 min after injection of TRH and T_4 (T_4 1 h before TRH)	130 min after injection of TRH and T_4 (T_4 2 h before TRH)
0,28±0,02 (7)	0,72±0,02 (6)	<0,015 (7)	0,49±0,03 (6)	0,40±0,03 (7)

Legend. Number of determinations in parentheses.

termined spectrophotometrically by the optical density of a solution of reduced neotetrazolium in the presence of paraphenylenediamine, was expressed in mg neotetrazolium/100 mg wet wet of tissue [6]. Immunoreactive thyrotropic hormone (TTH) was determined by the method described in [1].

EXPERIMENTAL RESULTS AND DISCUSSION

Cytochrome oxidase activity in the adenohypophysis of intact rats and of rats subjected to left-sided thyroidectomy 4 weeks before sacrifice (Table 1) was not significantly changed after the addition of 0.01 μg/ml TRH or 20 μg/ml T_4 to the incubation medium. This indicates that small doses of TRH and T_4, which affects the intensity of secretion of TTH and prolactin, can exert this effect without any visible changes in cytochrome oxidase activity. The sensitivity of the lactotrophs and thyrotrophs of the adenohypophysis to TRH and T_4 is known to be increased if thyroid function is blocked or considerably inhibited [8, 10]. The absence of sensitivity of the cytochrome system of adenohypophyses obtained from rats after partial thyroidectomy to small doses of TRH and T_4 can evidently be explained by the great powers of compensation of the thyroid gland. This is confirmed by investigations showing that the TTH concentration in the plasma and pituitary of thyroidectomized animals differs only a very little from that in intact rats [2]. Meanwhile, a 100-fold increase in the dose of TRH led to marked stimulation of cytochrome oxidase activity in intact animals (Table 1), showing that the activity of this enzyme depends on hypothalamic releasing hormone.

The considerably greater sensitivity of the enzyme to regulatory hormonal factors was found in the adenohypophyses of rats whose thyroid function had been blocked with methylthiouracil (Table 2). The basal level of cytochrome oxidase activity in the adenohypophyses of these animals was only half that in intact rats. Similar results with respect to glucose-6-phosphate dehydrogenase against the background of increased TTH secretion were obtained by Ochi et al. [5]. Cytochrome oxidase activity in the animals receiving methylthiouracil was increased by 1.5-2 times by both doses of TRH and was reduced by the action of T_4 in a dose of 20 μg/ml. The mechanism of potentiation of the response of the anterior pituitary to regulatory factors in hypothyroidism is evidently extremely complex. In particular, information has been obtained that additional secretory cells develop in the adenohypophyses of animals with T_3 and T_4 deficiency. The increased ability of the plasma membranes of the adenohypophyses of rats receiving methylthiouracil to bind labeled TRH [5] could be evidence of an initial change in the properties of the receptor apparatus of the thyrotrophs. The next experiments were carried out on the pituitary glands of rats receiving methylthiouracil.

Preincubation of the adenohypophyses with 20 μg/ml T_4 for 1 h before the addition of 1.0 μg/ml TRH to the samples prevented manifestation of the action of the TRH and the enzyme activity was the same as initially. Prolonging incubation with T_4 to 2 h led to a fall in enzyme activity to 50% below the control level. Actinomycin D, which blocks nucleic acid synthesis, had no significant effect on cytochrome oxidase activity, just as in experiments by other workers it did not affect $^{14}CO_2$ formation [11] and TTH secretion [3]. However, if the adenohypophyses were incubated successively for 2 h with actinomycin and for 1 h with 20 μg/ml T_4 and for 10 min with 1 μg/ml TRH, the cytochrome oxidase activity corresponded to the value obtained after treatment with TRH alone. The inhibitory action of T_4 on cytochrome oxidase activity was thus completely abolished by actinomycin D. In experiments in vitro an increase in the blood TTH concentration was observed in response to addition of TRH, but a sharp decrease was obtained in response to T_4 (Table 3). If injected into rats 1 and 2 h before TRH, T_4 largely inhibited the response of TTH to this specific stimulator.

The marked increase in cytochrome oxidase activity, accompanied by intensification of tissue respiration, was evidently necessary to provide the energy for the more rapid secretion of hormones. Since actinomycin D can prevent the inhibition of cytochrome oxidase by T_4, this suggests that T_4 induces the synthesis of an unknown protein substance that acts as TTH antagonist. A similar conclusion was drawn by other workers with respect to the effect of T_4 not only on tissue respiration [7, 8], but also on the secretion [3] and synthesis [9] of TTH.

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COMBINED ACTION OF PROTEIN-CHONDROITIN-4-KERATAN-SULFATE AND HYALURONIC ACID ON AGGREGATION AND ADHESION OF RED CELLS

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The rate and degree of aggregation of red cells produced by a mixture of protein-chondroitin-4-keratan-sulfate (PCKS) and hyaluronic acid (HUA) were found to be greater than the sum of the values of the corresponding indices for the separate action of these proteoglycans on red cell aggregation in the same concentrations as in the mixtures. It is suggested that this effect is due to the formation of a hybrid PCKS–HUA complex in the mixture which is more accurate as regards red cell aggregation than the separate components.
KEY WORDS: protein-chondroitin-4-keratan-sulfate; hyaluronic acid; complexes; red blood cells; aggregation.

Studies of the role of proteoglycans in the aggregation and adhesion of cells, using red blood cells as a model of isolated cells have shown that the ability of protein-chondroitin-4-keratan-sulfate (PCKS) and hyaluronic acid (HUA) to induce nonspecific reversible aggregation of red cells is due mainly to the property of these biopolymers of creating supramolecular complexes and three-dimensional structures in solutions which displace the red cells from the space they occupy into a separate phase. Electrostatic interaction between the red cell surface and these macropolyanions evidently plays a less important role in red cell aggregation [2, 3]. It has been suggested that red cell aggregation induced by PCKS and HUA is an expression of common properties of these proteoglycans of concentrating various tissue elements in a definite and limited space, and thus enabling all forms of interaction between them to be manifested [2, 3, 5].

In order to probe deeper into the role of proteoglycans in cell adhesion, in the investigation described below the combined action of PCKS and HUA on red cell aggregation was studied, for in many types of connective tissue these two substances coexist in various amounts and, by combining with each other, they may form hybrid complexes [6-10].

EXPERIMENTAL METHOD

PCKS was isolated from the cartilaginous rings of the bovine trachea [4] and HUA from human umbilical cords [1]. Both biopolymers were used in the experiments as their potassium salts.

Rabbit red blood cells were washed with physiological saline and a 1% (by volume) suspension of these cells was prepared in the same solution. Proteoglycans dissolved in 0.16 M NaCl were added to a known volume of the suspension in sufficient quantity to obtain the necessary final concentration of the proteoglycan. The mixture was quickly stirred and part of it transferred to a counting chamber, after which it was photographed at various time intervals under the microscope (magnification 120). The total number of cells was counted visually, by means of a projector, from the photographic frames taken during the first 1-2 min. The number of single red cells, i.e., unaggregated, was counted in the same frames obtained subsequently. The difference between the initial total number of red cells and the number of cells still remaining single at the subsequent times gave the number of red cells forming aggregates. Aggregation was expressed by the number of aggregated red cells as a percentage of their total number. A suspension of red cells in physiological saline, photographed after the same time intervals as the experimental samples, served as the control. Aggregation of the red cells was not observed in the control samples.

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