Inhibition of cyclic 3'5' monophosphate synthesis in rat testis by L-triiodothyronine

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Summary

Cytosolic adenylate cyclase activity from rat seminiferous tubules is inhibited by L-triiodothyronine (L-T3). In a typical dose-response curve, using Mn-ATP as substrate, no effect is observed at 10^{-10} M L-T3; about 15 to 25% inhibition is found in the range between 10^{-9} and 10^{-6} M L-T3 and finally a sharp enzyme inhibition is evident at increasing hormone concentrations from 10^{-6} to 10^{-4} M. Incubation of decapsulated testes with L-T3 leads to a decrease of intracellular cyclic AMP levels. Dose-response relationships for such effect are similar to those found for adenylate cyclase activity. In this case a clear response is observed at 10^{-8} M L-T3.

Abbreviations

DEAE-cellulose, diethylaminoethyl cellulose; TRIS, Tris (hydroxymethyl) aminomethane; and EDTA, ethylenediamino tetracetate

Introduction

Evidence on the role of cyclic nucleotides as mediators of thyroid hormone actions is rather confusing. Stimulation by thyroid hormones of adenylate cyclase in myocardial (1, 2) and spermatidic preparations (3) has been reported. An opposite effect was found by Friedman et al. (1977) in bovine thyroid membrane fractions (4). In this system, thyroid hormones, inhibited both basal and TSH-stimulated cyclase activities at unphysiological concentration. This inhibition could explain earlier findings on the ability of thyroid hormones to decrease intracellular cyclic AMP accumulation in thyroid gland (5, 6, 7). On the other hand, the inhibitory action of thyroid hormones and some polyhalogenated phenols on several Zn^{2+}-containing dehydrogenases has been described (8, 9).

As was pointed out by Braun and Dods (10) and confirmed by Neer (11), adenylate cyclase activity associated with rat seminiferous tubules has some relevant characteristics: 1) association to cytosolic proteins; 2) insensitivity to gonadotrophins; 3) insensitivity to fluoride and guanyl nucleotides; and 4) strong dependence on Mn^{2+}. This paper reports that thyroid hormones specifically inhibit this enzyme activity.

Materials and methods

Enzyme purification

Decapsulated testes from Wistar rats were homogenized in three volumes of an ice-cooled solution containing 50 mM Tris-HCl buffer, pH 7.5, 1 mM mercaptoethanol and 0.5 mM EDTA, using a SDT Tissuemizer (Tekmar Co) provided...
with 182 E shaft and generator. The homogenate was centrifuged at 7700 × g for 10 min and the supernate, containing most of the adenylate cyclase activity, was decanted. This fraction, was further purified by ultracentrifugation at 105,000 × g for 60 min and chromatography in DEAE-cellulose. The column (20 × 3 cm) equilibrated with the homogenization buffer, was loaded with 60 ml of the 'ultracentrifugation supernate' and washed with 150 ml of the same buffer solution; most of the non-absorbed proteins were removed in this form. Elution was performed with a NaCl linear gradient from 0 to 0.5 M (500 ml total volume) made in the same buffer solution. Fractions of about 15 ml were collected at a rate of 1.7 ml per min. Adenylate cyclase activity eluted as a single peak at about 0.25 - 0.30 M NaCl. Specific enzyme activity in the peak was 50 to 150 pmol per min per mg protein. The fraction thus obtained, referred to as 'DEAE preparation' was used as enzyme source in most of the experiments.

Adenylate cyclase assay

The standard incubation mixture contained 50 mM Tris-HCl buffer, pH 7.5, 0.2 mM 3-isobutyl-1-methyl-xanthine, 1 mM cyclic AMP, 2.5 mM MnCl₂, 0.5 mM [α-32P] ATP (specific activity 50 to 200 cpm per pmol), 2 mM phosphocreatine, 0.2 mg per ml creatine kinase and enzyme fraction (50 to 200 μg of protein). The volume was 0.1 ml. Incubations were performed at 37 °C for 3 to 10 min. Reactions were stopped and cyclic AMP in the samples was purified according to Salomon et al. (12).

Cyclic AMP assay in tissue samples

Duplicate half or quarter pieces of decapsulated testes were incubated at 37 °C by shaking in glass scintillation vials with 2 ml of Medium 199 (Gibco) adjusted to pH 7.4, and containing 0.1 mM 3-isobutyl-1-methyl-xanthine plus the indicated concentrations of L-T₃. Prior to the incubation, the vials were gassed with a O₂-CO₂ mixture (95:5, v:v). At the indicated times, the tissue samples were rapidly transferred to heavy wall glass centrifuge tubes (Sorvall; 13 mm I.D. × 100 mm length), containing 1 ml of ice-cooled 12% trichloroacetic acid and 25 pmol of [3H] cyclic AMP (about 5,000 cpm). The samples were then briefly sonicated in order to disperse the tissue. After centrifugation for 10 min at 6000 rpm, the supernatant fluids were extracted five times with five volumes each time of sulfuric ether and lyophilized.

The precipitates from the latter centrifugation were dissolved in 2 ml of 1 N NaOH and assayed for protein.

After lyophilization the material was resuspended in 2 ml of 50 mM sodium acetate buffer, pH 6.2. Duplicate aliquots (0.4 ml) of this resuspension were diluted in 5 ml of Bray's cocktail and counted for radioactivity. Recoveries were about 70%.

Aliquots of the resuspended lyophilized material were diluted in acetate buffer and subjected to acetylation according to the procedure of Frandsen and Krishna (13). Radioimmunoassay of acetylated samples and standards was performed according to Steiner et al. (14), using the kit purchased from New England Nuclear.

Analytical procedures

Protein was assayed according to Lowry et al. (15) using crystalline bovine serum albumin as standard.

Reagents

ATP, cyclic AMP, creatine phosphate, neutral alumina, L-triiodothyronine, thyroxine, triiodothyroacetic acid, L-monoiodotyrosine and L-diiodotyrosine were purchased from Sigma. Creatine kinase was from Boehringer, AG 50 W-X4 resin, 200-400 mesh, from Bio-Rad, DEAE-cellulose from Eastman, and 3-isobutyl-1-methyl-xanthine from Aldrich. [3H] Cyclic AMP was purchased from New England Nuclear. [α-32P] ATP was prepared as previously described (16).

Data reported, are the result of one representative experiment of at least three of the same type. Assays have been done in duplicate of triplicate samples.

Experimental

Responsiveness of different cyclase systems

The effect of L-T₃ on adenylate cyclase activity