ACTION OF THYROXINE ON PEROXIDATION OF LIPIDS IN MITOCHONDRIAL MEMBRANES

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In a concentration higher than $5 \cdot 10^{-7}$ M thyroxine delayed the development of chemiluminescence and reduced the rate of rise of the slow flash. The antioxidative activity of thyroxine was only a little less than the activity of the classical antioxidant α-tocopherol. In lower concentrations (of the order of $1 \cdot 10^{-8}$ M) thyroxine had a peroxidant action, which was most marked in the case of intact mitochondria incubated in phosphate medium in the presence of Mg^{2+} ions and was weaker in mitochondria treated with Ca^{2+} ions in hypotonic medium.

KEY WORDS: thyroxine; chemiluminescence; peroxidation; antioxidant; mitochondria.

The aspects of the action of thyroxine on mitochondria (MC) which have received most study are its effect on oxidative phosphorylation and swelling of these organelles [9, 11].

Considering that iodine, which can be liberated from the thyroxine molecule in MC, is a strong oxidizing agent and that thyroxine itself belongs to the group of phenolic compounds, most of which are active antioxidants [1, 2, 10], it was decided to study the effect of thyroxine on peroxidation of lipids in mitochondrial membranes, for the products of this process can affect the permeability of the membranes and the activity of enzymes in these organelles [1, 3].

EXPERIMENTAL METHOD

Mitochondria were isolated from the rat liver by differential centrifugation in medium containing 0.25 M sucrose and 10 mM Tris–HCl buffer, pH 7.4. Protein was determined by Lowry's method. Respiration of MC was estimated polarographically. Native MC with respiratory control in medium with succinate ranging from 3.5 to 6.0 were used in the experiments. Chemiluminescence of MC in the presence of Fe^{2+} ions was recorded on the apparatus described previously [4]. The concentration of malonic dialdehyde (MDA) in the samples was determined by the reaction with thiobarbituric acid [12]. The antioxidative activity of thyroxine was determined from the change in the index δ at the stage of exponential development of chemiluminescence [2] in media containing: a) 115 mM KCl, 10 mM KH_2PO_4, pH 7.4 (phosphate medium); b) 110 mM KCl, 10 mM KH_2PO_4, 5 mM $MgCl_2$, pH 7.4 (phosphate medium + Mg^{2+}); c) 120 mM KCl, 0.5 mM Tris–HCl buffer, pH 7.4 (phosphate-free medium). An aqueous solution of L-thyroxine ("ch. d. a." ["analytic"] grade, Reanal) in 0.01 M NaOH was used. The MC were incubated with thyroxine for 5 min, after which a solution of $FeSO_4$ (from $1 \cdot 10^{-3}$ to $3 \cdot 10^{-5}$ M) was added and the recording of chemiluminescence was started. The temperature in the 10-ml measuring cuvette was 37°C. The final protein concentration in the sample was 0.5 mg/ml. In one series of experiments the MC were treated with hypotonic incubation medium for 5 min, after which they were sedimented by centrifugation at 8000 rpm for 10 min, the supernatant was poured off, and the MC were incubated in phosphate medium for 5 min. In another series of experiments the MC were preincubated for 30 min in phosphate-free incubation medium with the addition of $2 \cdot 10^{-4}$ M $CaCl_2$; they were then sedimented by centrifugation (10 min at 8000 rpm) and, finally, treated with hypotonic medium.

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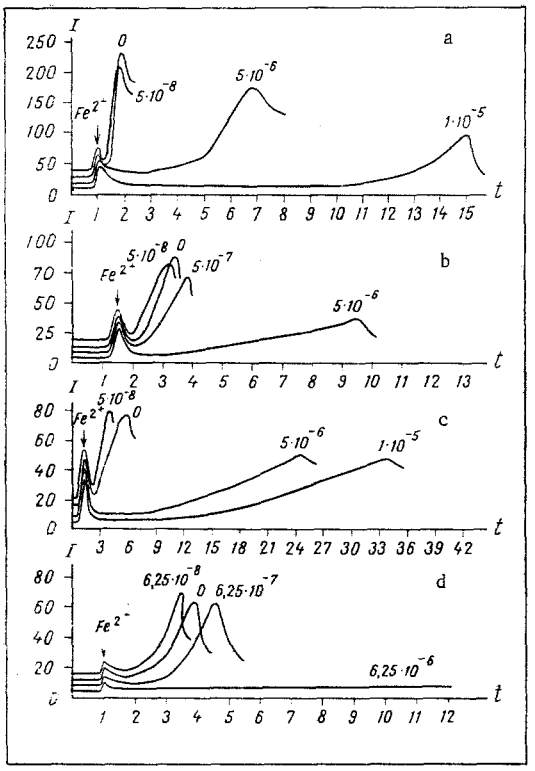

Fig. 1

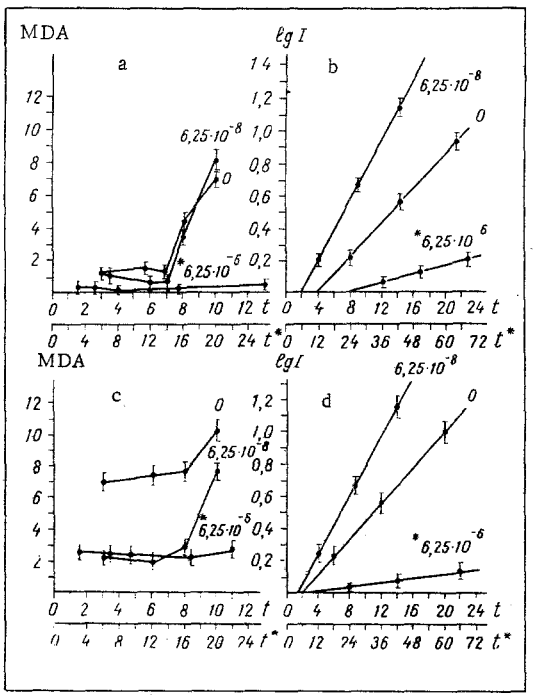

Fig. 2

Fig. 1. Effect of thyroxine on development of chemiluminescence of MC: a) intact MC; b) MC treated with hypotonic medium (10 mM Tris-HCl, pH 7.4); c) MC preincubated in hypotonic medium with Ca^{2+} ions ($2 \cdot 10^{-4}$ M); d) MC in incubation medium containing Mg^{2+} ions ($6.25 \cdot 10^{-5}$ M). Incubation medium: 115 mM KCl, 10 mM KH_2PO_4, pH 7.4; I) intensity of luminescence (relative units); t) time after addition of Fe^{2+} (in min). Numbers near curves indicate final concentrations of thyroxine (in M).

Fig. 2. Effect of thyroxine on accumulation of MDA (a, c) and rate of rise of initial stage of slow flash of chemiluminescence of MC (b, d). Incubation medium: a, b) phosphate containing Mg^{2+} ions ($6.25 \cdot 10^{-5}$ M); c, d) without Mg^{2+} ions. MDA) Concentration of malonic dialdehyde (in μmoles/mg protein); I) intensity of luminescence (in relative units); t) time (in min). Numbers near curves indicate final concentrations of thyroxine (in M).

EXPERIMENTAL RESULTS

It was shown previously that hormones with steroid structure, with a phenol ring and hydroxyl group, inhibit the peroxidation of lipids [2, 6-8]. The study of the kinetics of chemiluminescence of MC in the presence of Fe^{2+} ions in the various stages of the process can provide the answer to the question of the mechanism of the antioxidant action of a given agent, for the rate of the process at each stage is restricted by a definite reaction [1, 3]. Structural changes in the mitochondrial membranes themselves can also be reflected significantly in the development of chemiluminescence [5]. Considering that thyroxine affects membranes of MC, the kinetics of chemiluminescence was studied both in a suspension of intact MC, in MC subjected to hypotonic treatment, and also in MC preincubated in medium with Ca^{2+} ions. In some experiments the kinetics of chemiluminescence of MC was measured in phosphate medium containing Mg^{2+} ions.

As Fig. 1 shows, thyroxine in relatively low concentrations affected the development of chemiluminescence of MC by lengthening the latent period and depressing the slow flash. In concentrations of the order of 10^{-6} M thyroxine, the process of peroxidation in MC could be almost completely inhibited, as shown by the absence of the slow flash of luminescence (Fig. 1d) and the sharp decrease in accumulation of peroxidation products (Fig. 2a, c). It is interesting to note that in some cases the process of inhibition of peroxidation by thyroxine was accompanied by an increase in the intensity of the slow flash, indicating that thyroxine can sensitize luminescence. The ability of certain steroid hormones to sensitize chemiluminescence of MC was demonstrated previously [5].

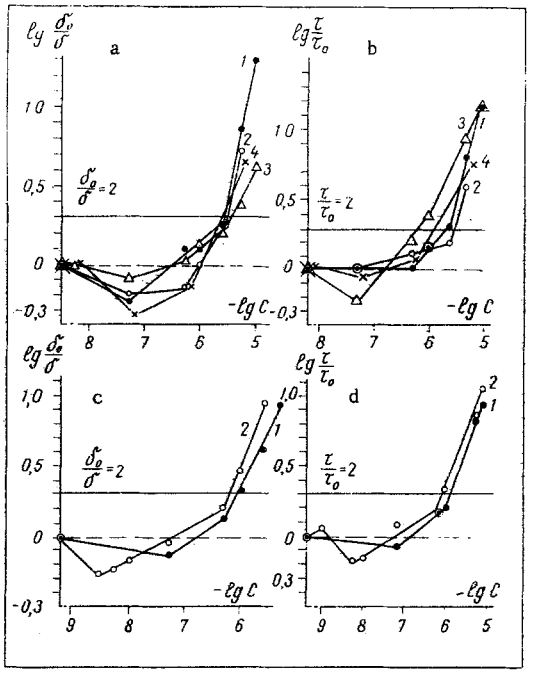

Fig. 3. Graphs showing log δ_0/δ and log τ/τ_0 as functions of thyroxine concentration in suspension of MC incubated in medium with (a, b) and without (c, d) phosphate: 1) intact MC; 2) MC incubated in hypotonic medium; 3) MC treated in hypotonic medium with Ca^{2+} ions ($2 \cdot 10^{-4}$ M); 4) intact MC in medium with Mg^{2+} ions ($6.25 \cdot 10^{-5}$ M); δ, δ_0) rate of rise of chemiluminescence in initial stage of slow flash in presence and absence of thyroxine, respectively; τ, τ_0) latent periods of development of luminescence, respectively; log C) concentration of thyroxine (in M).

For quantitative assessment of the antioxidative activity of thyroxine the index δ [1] was determined as the tangent of the angle of slope of the exponential part of the slow chemiluminescence flash (Fig. 2b, d), after which the concentration of thyroxine sufficient to double the δ_0/δ ratio was found graphically and the antioxidant activity was calculated by the equation $A = (\delta_0/\delta - 1)$ [InH], where [InH] is the concentration of inhibitor [2]. The antioxidative activity of thyroxine calculated in this way was 1.25×10^6 M^{-1}. Comparison with the activity of other antioxidants [2] shows that thyroxine is only a little inferior to α-tocopherol in its activity and is 1-2.5 orders of magnitude more active than the estrogenic steroid hormones. To rule out any possible role of swelling of MC in the mechanism of the antioxidative action of thyroxine, its activity also was estimated in a suspension of organelles subjected to preincubation in hypotonic medium with or without Ca^{2+} ions. Treatment of MC incubated in phosphate medium in this way had no significant effect on the index A ($3.6 \cdot 10^5$ M^{-1} compared with $1.25 \cdot 10^6$ M^{-1} for intact MC). In phosphate-free incubation medium a considerable difference was observed between the antioxidative activity of thyroxine on intact organelles ($A = 8.6 \cdot 10^5$ M^{-1}) and MC treated with hypotonic medium and Ca^{2+} ions ($A = 6 \cdot 10^9$ M^{-1}). In incubation medium containing Mg^{2+} ions the activity of thyroxine ($A = 4.5 \cdot 10^5$ M^{-1}) was close to the activity of the hormone in a suspension of intact MC.

Thyroxine also had a marked effect on the latent period (τ). The concentrations of the hormone lengthening the latent period of chemiluminescence and reducing the rate of rise of the slow flash were found to be similar in magnitude (Fig. 3), thus confirming the direct participation of thyroxine in the inhibition of free-radical oxidation of unsaturated fatty acids of the mitochondrial membrane.

The interesting fact was observed that in lower concentrations still (about $1 \cdot 10^{-8}$ M) thyroxine accelerated the development of the slow flash, shortened the duration of the latent period of chemiluminescence (Figs. 1 and 3), and increased the accumulation of MDA in the system (Fig. 2a, c), i.e., had a low degree of prooxidative activity which was manifested during incubation of the MC both in phosphate and Tris–HCl incubation media (Fig. 3). The prooxidative action of thyroxine differed in magnitude in the case of MC exposed to the action of disintegrating agents (hypotonic medium and Ca^{2+} ions), and in organelles incubated in isotonic medium in the presence of Mg^{2+}; this difference also depended on the presence of phosphate: In incubation medium containing

phosphate the prooxidative effect of thyroxine was higher on intact MC and in medium without phosphate on MC exposed to osmotic shock (Fig. 3). Activation of peroxidation by thyroxine was possibly somehow connected with its action on hydrolysis of phospholipids in the membrane [13] and subsequent swelling of MC [11], but this problem requires additional study.

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