ACTION OF ANTIOXIDANTS AND CHELATING AGENTS ON THYROXINE-INDUCED SWELLING OF MITOCHONDRIA

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The rate of swelling of rat liver mitochondria in the presence of thyroxine depended only a little on the cationic composition of the medium (KCl, NaCl, or choline chloride), which suggests an unselective increase in mitochondrial membrane permeability for cations in the presence of thyroxine. The antioxidant α-tocopherol and β-ionol, in concentrations completely suppressing peroxidation of lipids, did not affect thyroxine-induced swelling of the mitochondria, which is thus not connected with lipid peroxidation. The kinetics of swelling and its inhibition by the Ca-chelating agent EGTA are evidence that Ca^{2+} is essential for induction of this process. Thyroxine swelling of mitochondria is evidently based on activation of membrane phospholipase in these organelles.

KEY WORDS: *mitochondria; thyroxine; phospholipase; calcium; antioxidants.*

Uncoupling of oxidative phosphorylation and intensive swelling of the mitochondria *in vivo* and *in vitro* are the known effects of thyroid hormones at the subcellular level [7, 9, 10, 13]. These two effects of thyroxine or its analogs in mitochondria accompany one another in the same way as is observed, for example, during the uncoupling of oxidation and phosphorylation and swelling of mitochondria under the influence of valinomycin [7] or peroxidation of lipids [2, 14]. The possibility cannot be ruled out that the uncoupling of mitochondrial respiration induced by thyroxine is based on the swelling which precedes it [5, 12].

Thyroxine-induced swelling is by nature one of the active types of swelling [10]. Active swelling of mitochondria can be caused [8] by detergents, valinomycin, by activation of phospholipase A_2, or by induction of peroxidation (D, V, Ph, and P types of swelling respectively). These four types of swelling differ in certain indices, the most important of which are the character of the kinetics of the swelling process and the effect of antioxidants and of chelating compounds for Ca^{2+} and Mg^{2+} ions.

The object of this investigation was to study the types of active swelling of mitochondria induced by thyroxine.

EXPERIMENTAL METHOD

Thyroxine was used in the D and DL forms, which differed as regards neither the kinetics nor the degree of swelling induced by them. The concentration of the hormone in the samples was $4 \cdot 10^{-7}$-$6 \cdot 10^{-7}$ mole/mg protein. Mitochondria were isolated from rat liver in 0.25 M sucrose with 0.01 M Tris-HCl, pH 7.4 [4]. The degree of swelling of the mitochondria at 20°C was judged from the decrease in the scattering of light in the suspension, using an apparatus described previously [8].

EXPERIMENTAL RESULTS

As Figs. 1 and 2 show, thyroxine-induced swelling is characterized by a short latent period, reminiscent of all other types of Ph swelling, irrespective of whether due to the addition of detergent, fatty acids, or Ca^{2+} ions [8]. In this respect the effect of thyroxine differed sharply from the virtually instantaneous action of valinomycin and the first phase of action of detergent [8]. This suggests that during the action of thyroxine the increase in cationic conductivity does not take place immediately, but after activation of phospholip-

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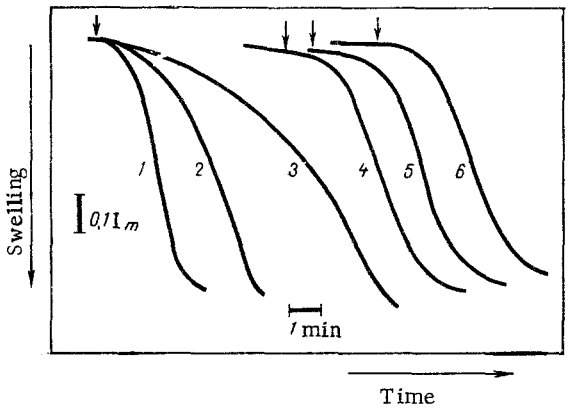

Fig. 1. Effect of concentration of thyroxine and of inhibitors of lipid peroxidation on swelling of mitochondria. Mitochondria (0.9 mg protein/ml) were suspended in medium containing 115 mM KCl, 1 mM $MgCl_2$, and 10 mM Tris-HCl, pH 7.4. The following were added: 1, 2, and 3) 60, 30, and 10 μM thyroxine respectively; 4) 60 μM thyroxine + 10 μM α-tocopherol; 5) 60 μM thyroxine + 1 μM β-ionol. Tocopherol and ionol were added to the incubation medium 1 min before thyroxine in concentrations completely inhibiting peroxidation of lipids. Time of addition of thyroxine shown by arrow next to each curve. I_m) Intensity of scattering of light of suspensions before addition of hormone.

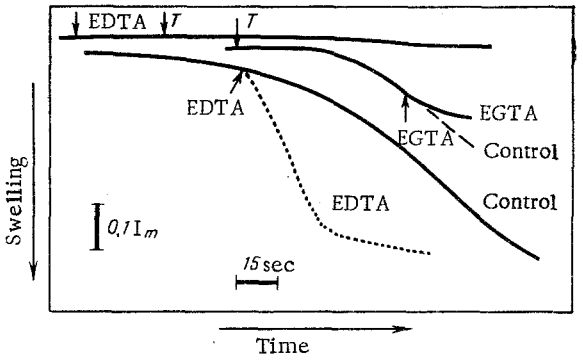

Fig. 2. Action of EDTA (1 mM) and EGTA (0.5 mM) on development of thyroxine (60 μM) swelling of mitochondria. T) Thyroxine. Remainder of legend as in Fig. 1.

ase. Evidence in support of this view was given by experiments to study the effect of the chelating agents EDTA and EGTA* on thyroxine-induced swelling. If EDTA was added before thyroxine, swelling was inhibited (Fig. 2), in agreement with the results of earlier observations [11], and probably attributable to the necessity for Ca^{2+} ions to activate the membrane phospholipase [15]. Addition of EGTA before the beginning of swelling inhibited it (Fig. 2), possibly on account of "blocking" of the cationic permeability channels formed under the influence of phospholipase, as a result of resynthesis of phospholipids in energized mitochondria. It is interesting to note that the addition of EDTA, which binds not only Ca^{2+}, but also Mg^{2+}, had the opposite action and caused a sharp increase in the rate of swelling (Fig. 2). This effect of EDTA was probably due not to acceleration of phospholipid hydrolysis, since Ca^{2+} also was bound, but to an increase in the cationic permeability of the modified membranes in the absence of Mg^{2+} ions. The stabilizing action of Mg^{2+} ions on membranes is well known [1, 3]. In the present case it can be postulated that phospholipase creates a local negative charge (or several charges in the case of its repeated action) at the site of hydrolysis, and this charge serves as the basis for the conduction of monovalent cations.

*Abbreviations: EDTA) ethylenediaminetetraacetic acid; EGTA) ethyleneglycolbis(β-aminoethyl ester)-N,N'tetraacetic acid.

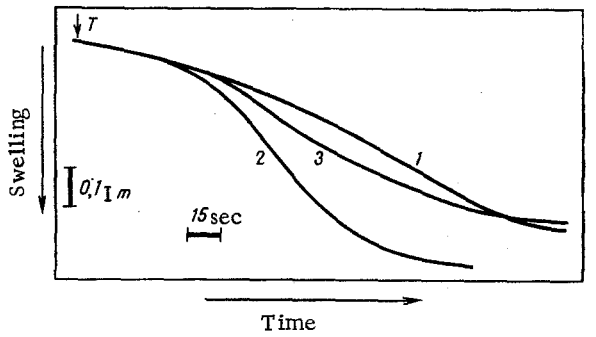

Fig. 3. Effect of electrolyte composition of medium on swelling of mitochondria induced by thyroxine (50 μM). 1) Composition of medium as in Fig. 1; 2) the same, but instead of KCl medium contains 115 mM NaCl; 3) the same, but instead of KCl medium contains 115 mM choline chloride. Remainder of legend as in Figs. 1 and 2.

Neutralization of this charge by Mg^{2+} leads to the blocking, whereas removal of Mg^{2+} from the membrane as the EDTA-Mg complex opens the channels once again. Such channels probably have negligible selectivity, which may depend on the chemical structure of the molecules hydrolyzed by the phospholipase. It has been shown, for instance, that thyroxine causes swelling of the mitochondria mainly in a medium containing potassium, but not in one containing sodium, on the basis of which it has been concluded that thyroxine has a valinomycin-like action on the mitochondria.

However, in these experiments the degree of swelling in medium with NaCl and choline chloride was at least not less than in medium with KCl (Fig. 3). In the light of the facts described above, this difference in the results was unimportant in principle.

It should be noted that α-tocopherol and β-ionol, inhibitors of lipid peroxidation, had no effect on thyroxine-induced swelling of the mitochondria (Fig. 1), as stated previously [4].

The physiological effect of thyroxine may thus be due to activation of mitochondrial phospholipase, which induces cationic permeability of mitochondrial membranes, active swelling of mitochondria, and uncoupling of oxidative phosphorylation.

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