TWO POSSIBLE MECHANISMS OF ACTION OF THYROXINE ON SWELLING OF MITOCHONDRIA

P. V. Sergeev, V. K. Fedorov,
V. M. Gukasov, and Yu. A. Vladimirov

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The effect of thyroxine on various types of swelling of the mitochondria (calcium-, detergent-, valinomycin-, and peroxide-induced) was studied. Under conditions favoring swelling of the mitochondria through phospholipase activation (a low concentration of Mg^{2+} ions, the presence of Ca^{2+} ions in the incubation medium) thyroxine causes swelling of the organelles to develop faster. When the phospholipase mechanism of swelling of the mitochondria is inhibited (high concentrations of Mg^{2+} ions), thyroxine considerably inhibits the development of swelling of the mitochondria induced by lipid peroxidation, by virtue of its antioxidant action.
KEY WORDS: thyroxine; phospholipase mechanisms; swelling; inhibition; mitochondria.

Swelling of the mitochondria (MC) is the most general response of their membranes to various harmful agents. Thyroxine swelling is of the active (energy-dependent) type [8], it has a certain induction period, and is inhibited by Mg^{2+} ions and by agents blocking electron transport [5]. There is as yet no general agreement regarding the mechanisms of thyroxine swelling [3]. According to one suggestion, it may arise through an increase in the permeability of the membranes due to iodine radicals (I^+) formed during the deiodination of thyroxine; to the blocking of high-energy intermediate products, with a consequent decrease in the ATP content; and to activation of hydrolysis of phospholipids with the formation of fatty acids [11].

One cause of swelling of MC both in vitro and in vivo may be peroxidation of lipids (POL) of biological membranes [1]. It was shown previously that thyroxine is an antioxidant (AO) and that, consequently, it should inhibit the swelling of MC due to peroxidation. On the one hand, thyroxine can thus evidently induce the swelling of MC and, on the other hand, it can inhibit it as an AO.

The object of this investigation was to study the conditions under which thyroxine can exert these two different actions.

EXPERIMENTAL METHOD

Mitochondria from rat liver were isolated by differential centrifugation in a medium containing 0.25 M sucrose and 0.001 M EDTA, pH 7.4, followed by washing to remove the EDTA. Protein in the MC suspension was determined by Lowry's method. Mitochondria with a respiratory control of the order of 3.5-6.9, determined polarographically, were used. The intensity of diffusion of light was measured with the FEK-59 photoelectric colorimeter and the output signal at a wavelength of 545 nm was recorded on the KSP-4 automatic writer. The intensity of the POL process during swelling of MC was judged from the accumulation of one of the end products of POL, namely malonic dialdehyde (MDA), by the reaction with thiobarbituric acid [9], and also by the change in chemiluminescence [2].

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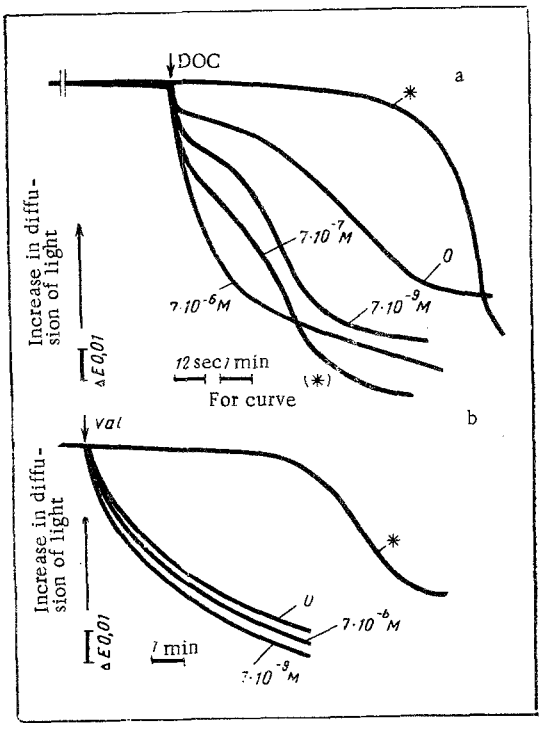

Fig. 1

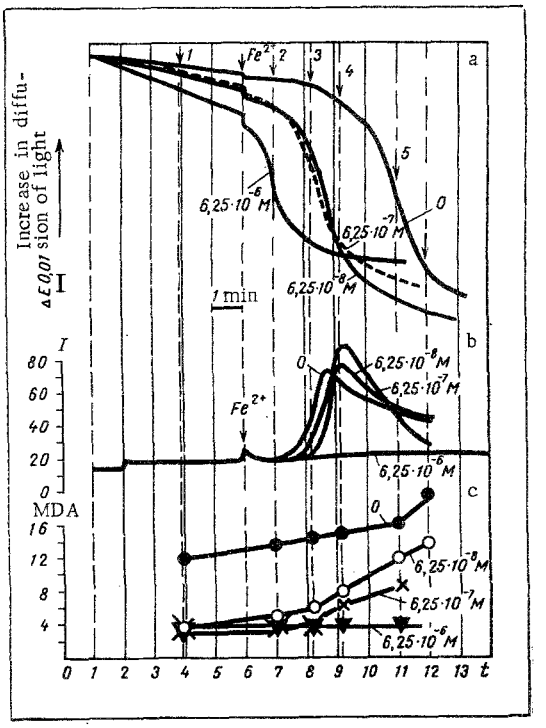

Fig. 2

Fig. 1. Effect of thyroxine on development of thyroxine on development of detergent- (deoxycholate – DOC) (a) and valinomycin (val)-induced (b) swelling of MC. Numbers by curves show final concentrations of thyroxine in MC suspension. Detergents and valinomycin added in final concentrations of $7 \cdot 10^{-5}$ and $1.4 \cdot 10^{-8}$ M, respectively. Curves marked by asterisk represent spontaneous swelling of MC in absence of hormone and modifiers. Composition of incubation medium: 115 mM KCl, 10 mM KH_2PO_4, 0.5 M succinate, pH 7.4; protein composition in suspension 2.5 mg/ml, T = 24°C.

Fig. 2. Effect of thyroxine on development of swelling (a), chemiluminescence (b), and MDA accumulation (c) in membranes of MC in magnesium-free incubation medium. Numbers by curves show final concentrations of hormone in MC suspension. t) Time of development of process (in min). MDA) Final concentration of malonic dialdehyde (in μmoles/mg protein). I) Intensity of chemiluminescence (in relative units). Arrows mark times of sampling for determining MDA during development of peroxide swelling of MC induced by Fe^{2+} ions added in a final concentration of $1.25 \cdot 10^{-4}$ M. Protein concentration in suspension 2 mg/ml, T = 24°C.

EXPERIMENTAL RESULTS

Swelling of MC can be induced by various agents, including inducers of cation transport (for example, valinomycin), detergents, Ca^{2+} ions (activating phospholipase), and substances activating POL [6, 7, 10]. Valinomycin swelling and the first phase of detergent swelling developed immediately after addition of these modifiers. Calcium swelling (the second phase of detergent and spontaneous swelling) develops after a certain time, which is evidently necessary for the activation of phospholipase by Ca^{2+} ions.

As Fig. 1b shows, if added at the same time as valinomycin to the suspension of MC, thyroxine had no appreciable effect on the development of swelling. This is explained by the fact that valinomycin created high permeability to cations, which is not affected by the hormone. As Fig. 1a shows, thyroxine does affect swelling induced by deoxycholate (DOC), speeding up the second phase connected with phospholipase activation; this effect is abolished by EDTA. The action of thyroxine was seen most clearly on spontaneous and peroxide swelling. The end result of the action of thyroxine in these cases depended on the concentration of Ca^{2+} and Mg^{2+} in the incubation medium of MC. In the absence of Mg^{2+} ions (Fig. 2) in the incubation medium the action of thyroxine was manifested as inhibition of POL, as shown by a decrease in the intensity of chemiluminescence and in the quantity of POL products of the MC membranes, i.e., thyroxine acted as an AO. However, under

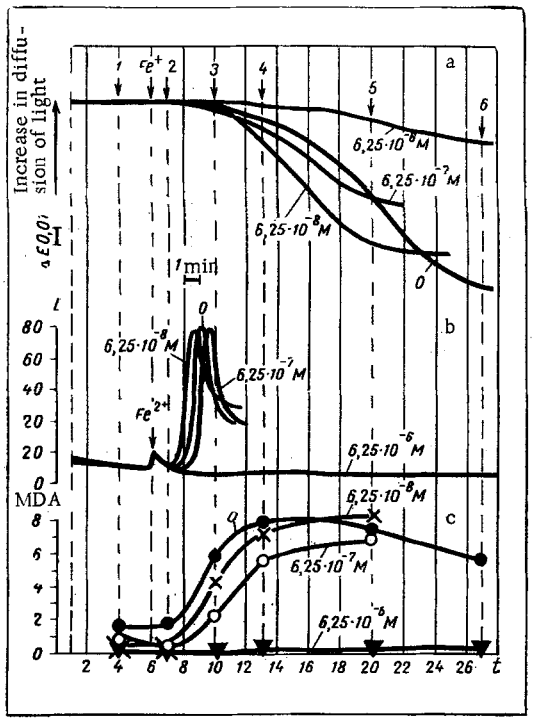

Fig. 3. Effect of thyroxine on development of swelling (a), chemiluminescence (b), and accumulation of MDA (c) in membranes of MC incubated in medium with Mg^{2+} ions. Composition of incubation medium: 110 mM KCl, 10 mM KH_2PO_4, 5 mM $MgCl_2$, pH 7.4. Legend as in Fig. 2.

these conditions thyroxine itself activated swelling to such a degree that the volume of MC had ceased to increase by the time that POL developed, and the latter made no additional contribution to swelling of the organelles. In the absence of Mg^{2+} ions the direct action of thyroxine (through phospholipase activation) exceeded its protective action (as an AO) on swelling of MC.

It will be clear from Fig. 3 that the action of thyroxine on swelling of MC was sharply altered in the presence of Mg^{2+} ions and of traces of Ca^{2+} ions in the incubation medium. Under these conditions thyroxine, in comparatively high concentrations, caused only slight and slow swelling of MC, although in a concentration of $6.25 \cdot 10^{-8}$ M thyroxine led to a greater increase in the rate of swelling and greater accumulation of MDA by the MC membranes than other concentrations of the hormone and also than in the control. The swelling observed in the presence of Fe^{2+} ions was due to POL. The rate of swelling of MC through activation of phospholipase in the presence of thyroxine (Ca^{2+} ions) was higher than after the development of POL. Inhibition of peroxide swelling of MC by thyroxine was accompanied by inhibition of POL, as manifested by a decrease in the intensity of chemiluminescence and in the accumulation of MDA.

Thyroxine can thus initiate or inhibit swelling of MC depending on its nature. When POL is negligible and the conditions are right for phospholipase activation (the presence of Ca^{2+} ions and low concentrations of Mg^{2+} in the system), thyroxine facilitates the swelling of MC. Conversely, if swelling of the mitochondria were caused by POL (the presence of POL catalysts, a high O_2 concentration) and provided that the Mg^{2+}/Ca^{2+} ratio were high, thyroxine, by inhibiting POL, would thereby stabilize the organelles. The possibility cannot be ruled out that the final effect of the biological action of thyroxine on MC depends on the state of the cell. Under conditions of oxygen deficiency (activation of phospholipases [4]) the hormone may aggravate the structural disturbances of the MC membranes. Under conditions of tissue hyperoxygenation (activation of POL) thyroxine may have a protective action on the membranes.

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