EFFECTS OF THYROID HORMONES ON INNER MITOCHONDRIAL MEMBRANE FLUIDITY

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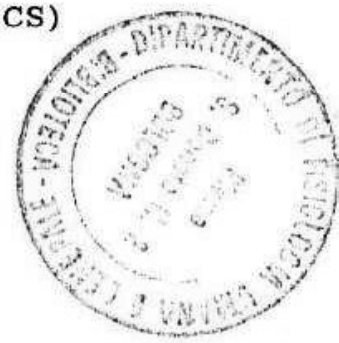

INTRODUCTION

Many authors have studied the effects of thyroid hormones and their diasteroisomers on mitochondrial activity (1,2). This topic is extensively debated because some authors have questioned the direct action of thyroid hormones on mitochondrial oxidative phosphorylation. Sterling et al. (3) purified an inner mitochondrial membrane protein that tighly binds thyroid hormones and demonstrated that this protein can interact with ADP-translocase. "In vivo" LT_3 affects both RNA and DNA polymerase activities in rat liver mitochondria and modified the mitochondrial population density in rat liver and myocardium (4,5). The same hormone enhanced proton pumping activity in inner mitochondrial membrane (IMM) as well as mitochondrial DNA polymerase activity (4). More recently De Giovanni et al. showed a positive mitochondrial effect on the activity of mitochondrial calcium transport (6).

Considering the present knowledge of inner mitochondrial structure authors have hypothesized that the direct action of thyroid hormones could be mediated by lipid membrane rearrangement, that is by modulation of inner membrane fluidity.

MATERIALS AND METHODS

Eighty day old male rats, Wistar strain, were used. They were killed under ether anaesthesia. The isolation and purification of mitochondria from rat liver was performed by differential centrifugation using the

Greenawalt's method (7). The mitochondrial protein concentration was determined using Lowry's method (8). The buffer (SMH): sucrose (70 mM) (Merck, Germany), mannitol (220 mM), HEPES (2 mM) (Calbiochem, Behring Corp. Germany), BSA (0.0073 mM) (Fluka, Germany) was used. Inner mitochondrial membranes were separated by lysing (9) the outer membrane with digitonine (1.1 mg/ml final concentration) (Calbiochem-Behring Corp. Germany) and sedimenting the inner membranes at 12000 rpm for 10 minutes in the same buffer. Fluidity was studied by spectrofluorometric method on ISS apparatus (ISS inc. Illinois, USA). The fluorescent label DPH was studied by means of Arrhenius plots of fluorescence versus temperature and Perrin relationships of polarized fluorescence (1/P) with T/η ratio. This parameter is inversely correlated to membrane fluidity according to the method of Ricchelli and Salvato as described by J.A. Gally et al. and L. Brand et al. (10,11). DPH final concentration was 0.32 mM, IMM final concentration by protein content was 5 mg/ml; the spectrofluorimetric data were obtained with λ_{exc}= 350 nm and λ_{em}= 430 nm. The hormones L-3,5,3'-triiodothyronine (LT_3), L-3,5,3',5'tetraiodothyronine (LT_4), their diasteroisomers and 3,5 diiodothyronine (LT_2) (Calbiochem-Behring Corp. Germany) were dissolved in buffer SMH without BSA.

RESULTS

Arrhenius plots show a decreased fluorescence quenching in the presence of both T_3 and T_4 isomers (fig. 1). The maximum effect is observed with 2 nM LT_3. Data for the other three isomers are less significantly influenced. In the presence of LT_2 we observe a more intensive fluorescence quenching at 1nM concentration.

The Perrin plots (fig. 2) show a generalized decrease of 1/P with L and DT_3 with a minimum at 4 nM. Similar effects are seen with LT_4 and only a slight increase of 1/P at 1 nM for DT_4 is evident. Data indicate a lowering effect on 1/P of LT_2 at 2, 4, 6 nM concentrations. The described studies show a decrease of fluorescence quenching in the Arrhenius plots (12). The effect is the highest at 2 nM LT_3 and at 1 nM LT_2.

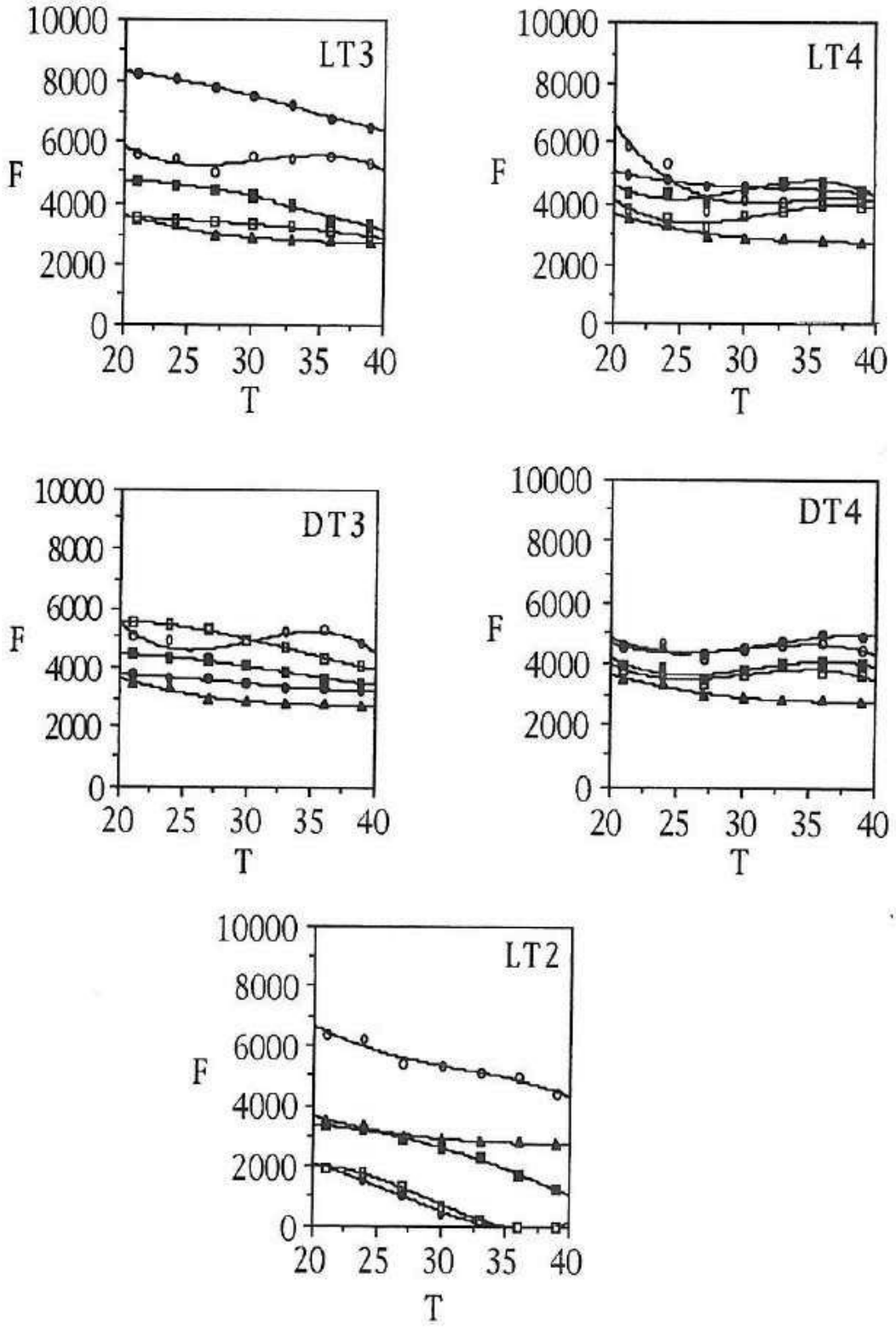

Fig. 1 - Effect of thyroid hormones and their analogues on the quenching of DPH (0.32 mM final concentration) fluorescence in IMM (5 mg/ml final concentration). (Δ = standard; ○ = 1nM; ● = 2nM; □ = 4 nM; ■ = 6nM.

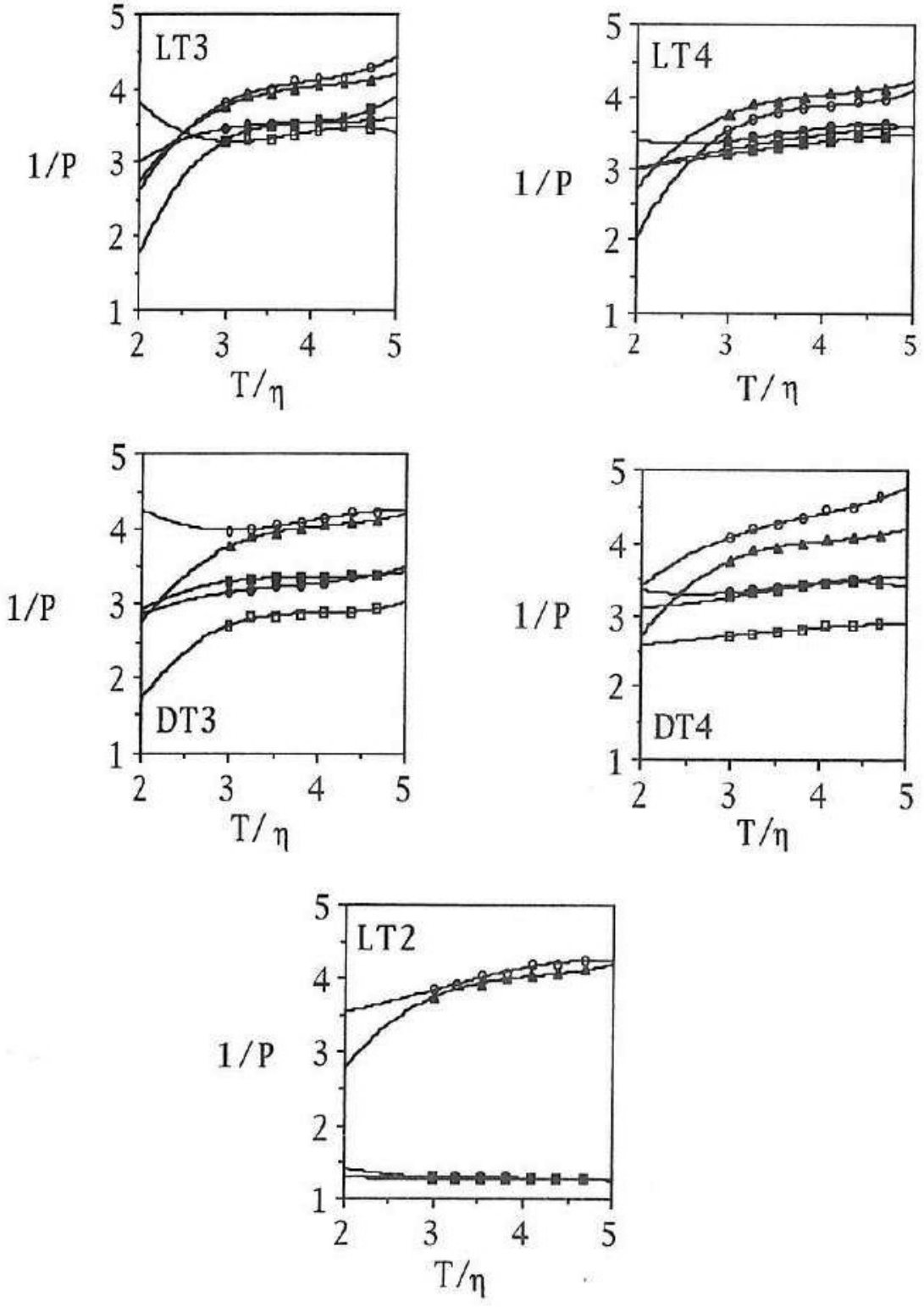

Fig. 2 - Perrin plots of DPH (0.32 mM final concentration) interaction with IMM (5mg/ml final concentration) P: fluorescence polarization; T: absolute temperature; η: intrinsic viscosity of water at the same experimental temperature. (Δ = standard; ○ = 1nM; ● = 2nM; □ = 4 nM; ■ = 6nM.

The studies on fluorescence polarization show a decrease of 1/P parameter for all the studied compounds, and the effect is particularly intensive for LT_2 at 2, 4 and 6 nM.

DISCUSSION

All the described results indicate that LT_3 and LT_4 have selective effects at concentrations similar to physiological tissue ones.

When the mid area of IMM is concerned, with the label DPH, the most intensive effects on the fluorescence parameters are shown by LT_2 (at 2,4,6 nM) but also by LT_3 even if less markedly, according to the affinity with the demonstrated receptors in IMM (13).

Comprehensively all the studied parameters confirm that many factors influence the interactions of thyroid hormones with IMM: the number of iodine atoms, that is their liposolubility, the L or D conformations, the affinity with IMM thyroid hormones receptors (13).

The balancing among all the described parameters defines the efficacy of the thyroid compound on the binding with the selective areas of the IMM. The most intensive influence on IMM metabolic activities well correlates with these data, as also shown by Buchanan et al. and Goglia et al. and us (6,13,14).

The LT_2 and LT_3 that interact with the central portion of IMM are also better bound by IMM receptors for thyroid hormone and demonstrate the most intensive efficacy on IMM enzyme activities.

Authors studied the effects of thyroid hormones and their diasteroisomers and 3,5-diiodothyronine (LT_2) on the fluidity properties of inner mitochondrial membrane (IMM) by specifical fluorescent probe for the internal zone of biological membranes, the 1,6-diphenyl-1,3,5-hexatriene (DPH). The studied parameters are Arrhenius and Perrin plots. The DPH shows a decreased fluorescence quenching in the presence of both T3 and T_4. The maximum effect is observed with 2 nM LT_2. LT_2 is more effective than LT_3 in the central zone. The data confirm the selective action of LT_3 and LT_4 on IMM fluidity.

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