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Thyroid Hormones Affect the Membrane Dipolar Organization. Is It a General Event in Their Non-genomic Action?

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Abstract. The surface balance technique was employed to study the interactions of 3,5,3′,5′ tetraiodo L-thyronine, 3,5,3′ triiodo L-thyronine, and 3,5-diiodothyronine with monomolecular phospholipid monolayers spread at the air-water interface. With this technique the insertion of thyroid hormones into egg yolk phosphatidylcholine was investigated. An increase of surface pressure and a substantial decrement in surface potential were observed after the injection of these hormones beneath a phospholipid monolayer. The negative dipole contribution upon hormone interaction opposes the well-known positive contribution of phospholipids. These effects correlated with iodo content of the thyroid molecule analogues 3,5,3′,5′ tetraiodo L-thyronine >3,5,3′ triiodo L-thyronine >3,5-diiodothyronine. To our knowledge, these observations suggest a new and surprising effect of thyroid hormones on the regulation of transmembrane dipolar organization.

Key words: Thyroid hormones — Membrane potential — Lipid monolayers — Non-genomic mechanism

Introduction

In the past, iodothyronines other than 3,5,3′ triiodothyronine (T_3) and thyroxine (T_4) present in the biological fluids have been regarded as inactive. However, a growing number of studies have indicated that 3,5-diiodothyronine (T_2) may have physiological relevance, acting principally at the level of mitochondria (Host, Rohos & Seitz, 1989; O'Reilly & Murphy, 1992; Goglia et al., 1994; Moreno et al., 1997; Arnold, Goglia & Kadenbach, 1998; Lombardi et al., 1998). It was generally accepted that, although T_4 is the principal secretion product of the thyroid gland, its metabolic and developmental effects are all mediated by T_3, which exerts its action at the level of its specific nuclear receptor sites (Oppenheimer, 1991; Chin, 1992; Tsai, 1994; Hulbert, 2000). Also, there is evidence that the thyroid hormones are normal constituents of biological membrane in vertebrates and, moreover, some physiological effects of these hormones actually occur at the membranes (*see* recent review of Hulbert, 2000). These hormones are strongly associated with membranes in tissues and normally rigidify them by affecting their lipid composition. It has been suggested that both effects exerted on membranes, either on their physical state or changes in their composition, are the result of several non-genomic thyroid hormone effects (Hulbert, 2000).

The interaction of thyroid hormones with lipid membranes was documented previously. To gain knowledge of the non-genomic mechanisms, previous work from our laboratory has demonstrated the transmembrane diffusion of thyroid hormones, using liposomes as a membrane system (Chehin et al., 1995, 1999; Farias et al. 1995). Several years ago, Lai and Cheng (1982, 1984), using electronic spin resonance techniques (EPR), reported that the lateral diffusion of the spin-labeled T_3 or T_4 inserted into liposomes was similar to the lateral diffusion of spin-labeled fatty acid. Additionally, EPR measurements using 5- or 10-deoxyl-stearate spin-labeled liposomes indicated also that the T_4 can penetrate the lipid core region of the liposomes (Rufini, S., Polizio, F. and Farías R.N. unpublished data), suggesting that both α and β hydrophobic rings are close to the lipid core, whereas the more hydrophilic and ionizable region of the T_4

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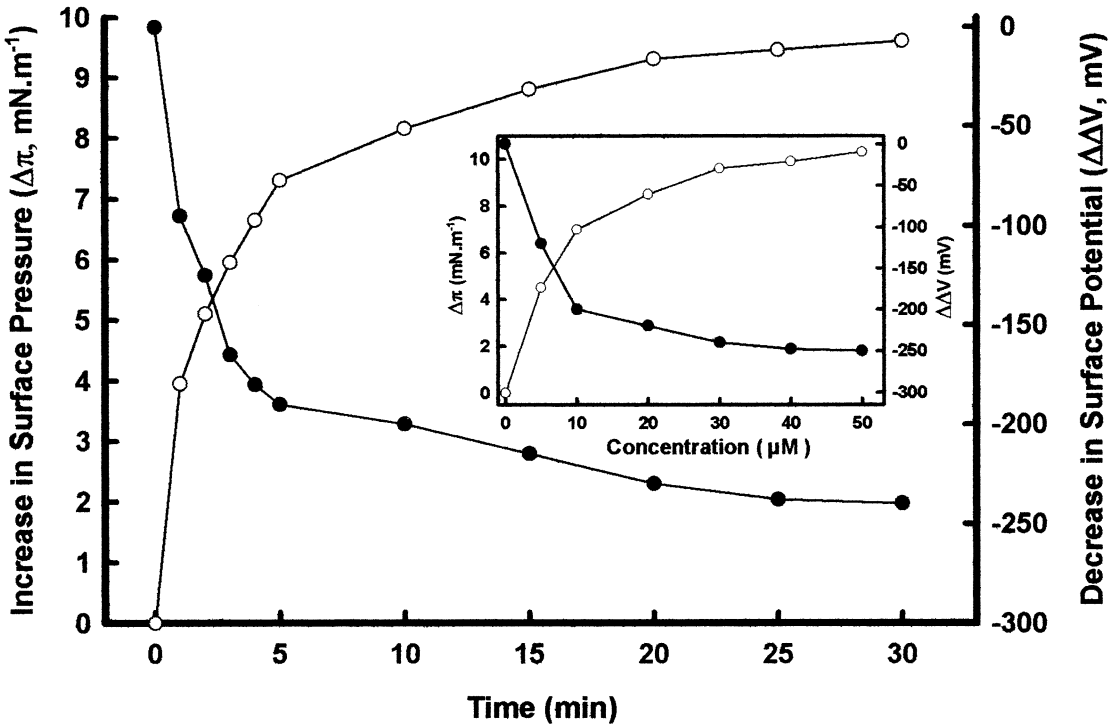

Fig. 1. Interaction of T_4 with phospholipid monolayers. Representative curve of time course of changes in surface pressure $\Delta\pi$ (○) and surface potential $\Delta\Delta V$ (●) after injection of an initial concentration of 30 μM of T_4 into the subphase of a PC monolayer set at an initial surface pressure of 20 $mN \cdot m^{-1}$. *Insert*: Dose dependence of the surface-pressure increase ($\Delta\pi$, ○), and surface potential ($\Delta\Delta V$, ●) induced by injection of T_4 at the initial concentration indicated. The values were collected at t = 30 min. The initial pressure of the PC monolayer was 20 $mN \cdot m^{-1}$ and the initial surface potential was 370 mV. Subphase, 50 mM phosphate buffer, pH = 7.4.

formed by the amino group and the carboxyl group of the alanine residue, is at the membrane/aqueous interface.

On the other hand, comparison of Raman spectra of pure T_4 with a 1:5 mole relation of hormone:egg-yolk phosphatidylcholine mixture reveals differences due to structural changes of T_4 induced by lipid-hormone interaction (Alvarez et. al., 2002). Here, we strengthen all these previous observations that reported a novel effect of the thyroid hormones on the membrane potential by studying the spontaneous incorporation of thyroid molecules into an artificial membrane system (lipid monolayers).

Materials and Methods

Materials

T_3, T_4, and T_2 were purchased from Sigma (St. Louis, MO) and used without further purification. Egg-yolk phosphatidylcholine (PC) was obtained from Avanti Polar Lipids (Birmingham, AL). Phospholipid preparations were dissolved in chloroform-methanol (2:1, v/v) under nitrogen and stored at −20°C. The phospholipid concentrations of the stock solutions were determined by phosphate analysis according to Ames, 1966. Thin-layer chromatograms of PC using a variety of solvent systems did not reveal any impurities.

Lipid Monolayer Experiments

Interaction between PC and thyroid hormones was studied by measuring the increase in surface pressure and changes in surface potential after injecting the hormone into the subphase beneath a lipid monolayer brought to a specified surface pressure. Adsorption measurements were performed in a Teflon trough of 20 ml in volume and 16 cm^2 in surface area, equipped with magnetic stirring. PC was spread at the air-water interface from chloroform-methanol with a microsyringe, allowing the solvent to evaporate before adjusting the lipid monolayers to the desired initial surface pressure. Once the phospholipid monolayer was set to the desired initial surface pressure (π_i), an amount of concentrated thyroid hormone analogues dissolved in a small volume (maximum 20 μl) to give a final concentration ranging from 10 to 50 μM, was injected into the subphase beneath the lipid monolayer-covered surface through an injection septum. Surface pressure measurement was performed with an LM 600 Beckman electronic microbalance and the surface potential was evaluated by ^{241}Am air ionizing and calomel electrode pairs connected to a millivolt meter (Fidelio, Maggio & Cumar, 1982). The absence of surface-active impurities from solvents and from the subphase solution was demonstrated by small changes in surface pressure and surface potential (≤0.1 $mN \cdot m^{-1}$ or < 5 mV) that occurred when a lipid-free interface was compressed. Subsequent changes in surface pressure (π) and surface potential (ΔV) at constant area, after thyroid hormone injection, were automatically recorded as a function of time. The subphase (50 mM sodium phosphate buffer pH 7.4) was continuously stirred with a Teflon-coated stirring bar. The subphase temperature was maintained by a water circulation bath at 25°C. Final values (π_f and ΔV_f) of interaction were taken when the

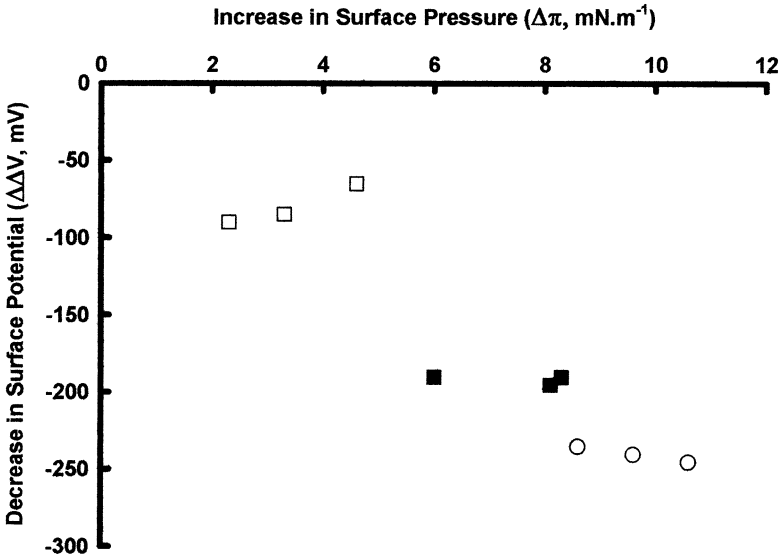

Fig. 2. Correlation of thyroid hormones interaction with the decrease in the surface potential of the phospholipid interface. Changes in surface pressure $\Delta\pi$ versus decrease in surface potential $\Delta\Delta V$ obtained for T_4 (○), T_3 (■), T_2 (□). The initial pressure of the PC monolayer was 20 $mN \cdot m^{-1}$. Subphase, 50 mM phosphate buffer, pH = 7.4. The initial hormone concentration was 30 μM. The values were collected at t = 30 min The values represent three independent experiments for each thyroid hormone.

changes in surface pressure and surface potential were less than 0.05 $mN \cdot m^{-1} min^{-1}$ and 1 $mV \cdot min^{-1}$, respectively (Fidelio et al., 1982). Changes in surface pressure and in surface potential upon hormone interaction are defined as $\Delta\pi = \pi_f - \pi_i$ and $\Delta\Delta V = \Delta V_f - \Delta V_i$, respectively. Average values were performed at least in triplicate. Reproducibility was ± 0.8 $mN \cdot m^{-1}$ in surface pressure and ± 5 mV in surface potential.

Results and Discussion

Lipid monolayers have previously been used by us to study the interaction of different proteins with lipids (Fidelio et al., 1981, 1982): the peptide hormones α-MSH and substance P with gangliosides (Gonzalez et al., 1996) and fatty acyl-coenzyme A and fatty acylcarnitines with phospholipids (Requero et al., 1995). Subsequent changes in surface pressure (π) and surface potential (ΔV) can be taken as a measure of penetration and interaction of thyroid hormone analogues with lipid monolayers. The surface-active properties of the thyroid hormone analogues were first tested in absence of phospholipids. None of the checked thyroid hormone analogues displayed surface activity by themselves in absence of phospholipid, i.e., no change in surface tension was observed with any of the hormones tested when injected into the subphase beneath a lipid-free interface. In comparable experiments using lipid monolayers and bilayers, several authors have arrived at the conclusion that the estimated equivalent lateral surface pressure in a biological membrane ranges from 20 to 30 $mN \cdot m^{-1}$ (Van Deenen et al., 1976; Israelachvili, Marcelja & Horn, 1980). To perform the present study, the PC monolayer was spread at an initial surface pressure of 20 and 30 $mN \cdot m^{-1}$. Figure 1 shows a typical time-course recording of the changes in the surface pressure ($\Delta\pi$) and surface potential ($\Delta\Delta V$) as a function of time, after the injection of 30 μM of T_4 into the subphase when the monolayer of PC was spread at an initial surface pressure of 20 $mN \cdot m^{-1}$. The penetration was rapid and, at 5 min or before, the changes were 60–80% of the final values. The rapid increase in surface pressure indicated that T_4 interacts with phospholipids and that it is markedly stabilized by the amphiphilic interface, since the hormone is able to support higher lateral packing only if lipid is present at the surface. After 30 min, an increase of surface pressure to about 10 $mN \cdot m^{-1}$ parallels a maximum decrease in surface potential of −250 mV (from about 370 mV to 120 mV), (Fig. 1). The interaction of T_4 hormone with lipids was dose-dependent and reached a plateau at 30 μM of initial concentration (Fig. 1, insert). Similar results were obtained after the adsorption of T_3 and T_2 to the PC monolayer, but the changes produced in both surface parameters were of minor magnitude. The changes attained for T_3 and T_2 in $\Delta\pi$ and $\Delta\Delta V$ were about 8 $mN \cdot m^{-1}$ and −200 mV and 3 $mN \cdot m^{-1}$ and −80 mV, respectively. A correlation between $\Delta\pi$ and $\Delta\Delta V$ is observed (Fig. 2). When the PC monolayer was spread at an initial surface pressure of 30 $mN \cdot m^{-1}$, the values of $\Delta\pi$ and $\Delta\Delta V$ were lower than at 20 $mN \cdot m^{-1}$. At 30 $mN \cdot m^{-1}$, the changes attained for T_4, T_3 and but T_2 in $\Delta\pi$ and $\Delta\Delta V$ were about 8 $mN \cdot m^{-1}$ and −170 mV, 5.5 $mN \cdot m^{-1}$ and −130 mV, and 1.0 $mN \cdot m^{-1}$ and −60 mV, respectively.

In monolayer experiments at constant area, the increase in surface pressure upon the addition of an amphiphile into the subphase is interpreted in terms of physical penetration of at least part of the interacting molecule into the lipid film (Quinn & Dawson, 1969; Fidelio et al., 1981; Pilon et al., 1987; Mustonen et al., 1993). The interaction observed for the different thyroid hormone analogues measured as penetration (surface pressure increase) correlates with the extent of reduction in surface potential. In turn, the different capability of thyroid hormones to decrease

the initial surface potential of the phospholipid monolayer correlates with the iodo content of the thyroid molecule analogues: $T_4 > T_3 > T_2$.

At pH 7.4, approximately 80% of the T_4 molecules (pK 6.73) have the phenolic hydroxyl group in the ionized form, whereas only approximately 10% of T_3 molecules (pK 8.45) have an ionized phenolic hydroxyl group (Korcek & Tabachnick, 1976). The T_2 molecules have a pK = 10. Thus, the pK values of the phenolic group of the iodothyronine do not correlate with changes in the surface pressure ($\Delta\pi$) and changes in surface potential ($\Delta\Delta V$) observed in the lipid monolayer experiments.

The negative surface potential developed at the interface after the thyroid hormone analogues' interaction implies that the dipolar organization of thyroid hormones at the interface is of a sign inverse to the dipolar organization of phospholipids. The surface potential of the interface is sensitive to the concentration and dipolar properties of the film-forming molecules. As a consequence of the amphipathic nature of phospholipids, the dipole moment of the chemical groups at the surface is asymmetric, given a total resulting surface dipole moment perpendicular to the interface that includes hydrocarbon tails, polar head group and polarized surface hydration water molecules (*see* recent review of Brokman, 1994). The ΔV of a phospholipid monolayer at 20–30 $mN \cdot m^{-1}$ is about 370–400 mV, with the positive end towards the air (Brokman, 1994). As a consequence of the hormone interaction, the surface potential decreases, making the surface potential of the interface less positive. Thus, the surface potential ΔV is an additive property of the interface and it is the consequence of the contribution of all surface dipoles: lipid plus hormone at the surface. Since thyroid hormones have no surface activity by themselves, the hormone-lipid interaction may be stabilized by the opposite resultant of the overall dipole moment of each molecule perpendicular to the interface. This property could be of physiologic importance, since thyroid hormones emerge as interfacial potential regulators of the dipolar organization of the cell membranes. Further research on our novel observation is necessary.

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