THYROID HORMONE ACTIONS AND MEMBRANE FLUIDITY

Blocking action thyroxine on triiodothyronine effect

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1. Introduction

Previous reports showed correlations between the cooperative behavior of mammalian and bacterial membrane-bound enzymes and membrane fluidity [1–3]. These correlations raised the possibility of evaluating changes in the membrane fluidity through changes in the cooperativity of these enzymatic systems. The application of Hill coefficient measurements as a test for membrane structure has been discussed [4] and the thermodynamic considerations reported [5]. Very small changes in the membrane–enzyme interaction, of the order of 700–800 cal/mol, would be enough to give a significant change in the values of n (Hill coefficient).

In this report, the hormonal action of L-3,5,3′-triiodothyronine (T_3) and L-thyroxine (T_4) at strict physiological levels on changes of the membrane fluidity evaluated through changes in the Hill coefficient of mammalian and bacterial membrane-bound enzymes is studied. In addition, a new blocking effect of T_4 on T_3 action is shown. Part of the present work was reported in preliminary form [6].

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2. Materials and methods

2.1. *Rat erythrocyte membrane enzymes*

Male Sprague-Dawley rats, grown after weaning on basic diet supplemented with 5% lard or corn oil, were used to obtain erythrocyte membrane exhibiting low or high fatty acid fluidity, respectively [1]. Blood samples were taken after 15 weeks by heart puncture. Procedures to prepare the erythrocyte membrane and to obtain the soluble form of acetylcholinesterase were described [1,7]. Acetylcholinesterase activity was determined according to a method reported [1]. The measurement of ATPase activities under initial velocity conditions and the calculation of the kinetic parameters for the inhibition by F^- of the membrane-bound $(K^+ + Na^+)$ATPase were described [1,8]. The reaction was stopped by the addition of 0.1 ml 5% sodium dodecylsulfate to 1 ml ATPase reaction mixture as described [9]. No turbidity was noticeable.

2.2. Escherichia coli *membrane enzyme*

The bacterial strain used was *E. coli* K-12 M_1 (*pho*$^-$, alkaline phosphatase). The bacteria was grown aerobically in a gyratory shaker at 20°C or 37°C in nutrient broth medium to obtain membrane preparation exhibiting low or high fatty fluidity [10]. An increase in the unsaturated fatty acid levels by lowering the temperature at which the bacteria is grown has been found [11]. Cells were harvested by centrifugation in mid-exponential phase. Preparations of the membrane

and soluble enzyme, the (Ca^{2+})ATPase assays, as well as the calculation of the kinetic parameters were described [10]. For the assay of the inhibition by Na^{2+} of the (Ca^{2+})ATPase, the reaction mixture consisted of 20 mM Tris–HCl buffer (pH 9.0), 1 mM $CaCl_2$, 2.5 mM Tris–ATP, 1 mM cysteine and increasing amounts of NaCl.

2.3. *Others*

T_3, T_4 were dissolved in some drops of 0.1 N NaOH. Thereafter, the stock solution was prepared and stored frozen until used. For the enzymatic assays appropiate dilutions from stock solutions were made.

3. Results

3.1. *Effect of T_3 and T_4 on allosteric inhibition by F^- of membrane-bound acetylcholinesterase*

A high correlation (r 0.90) was reported between the values of n for the allosteric inhibition by F^- of erythrocyte membrane-bound acetylcholinesterase and the membrane fluidity expressed as the ratio double-bound index to saturated fatty acids using rats fed a fat-free diet and rats fed with different fat-supplemented diets [1]. An increase in the fatty acid fluidity of the membrane was accompanied by a parallel increase in the cooperativity of the enzyme. Furthermore, the role of fatty acid composition in the changes of n values of this enzyme was confirmed by in vitro recombination experiments [12]. To study the actions of T_3 and T_4 on the erythrocyte membrane fluidity two synthetic diets (not deficient in essential fatty acids and producing maximal differences in membrane fatty acid fluidity) were chosen: lard- and corn oil-supplemented diets [1]. When the specific activity of acetylcholinesterase is plotted against the concentration of F^-, the shape of the curves are different between both groups. The values of n were 1.6 for corn oil-fed rats and 1.0 for lard-fed rats, in agreement with [1].

3.1.1. Corn oil-fed rats

As it can be observed in fig.1A in the presence of T_3, 1×10^{-9} M, the shape of the curve and values of n of corn oil-fed rats became equal to that of lard-fed rats. Figure 1B shows the blocking effect of T_4 on T_3 action. Values of 1.6 were obtained in the presence of

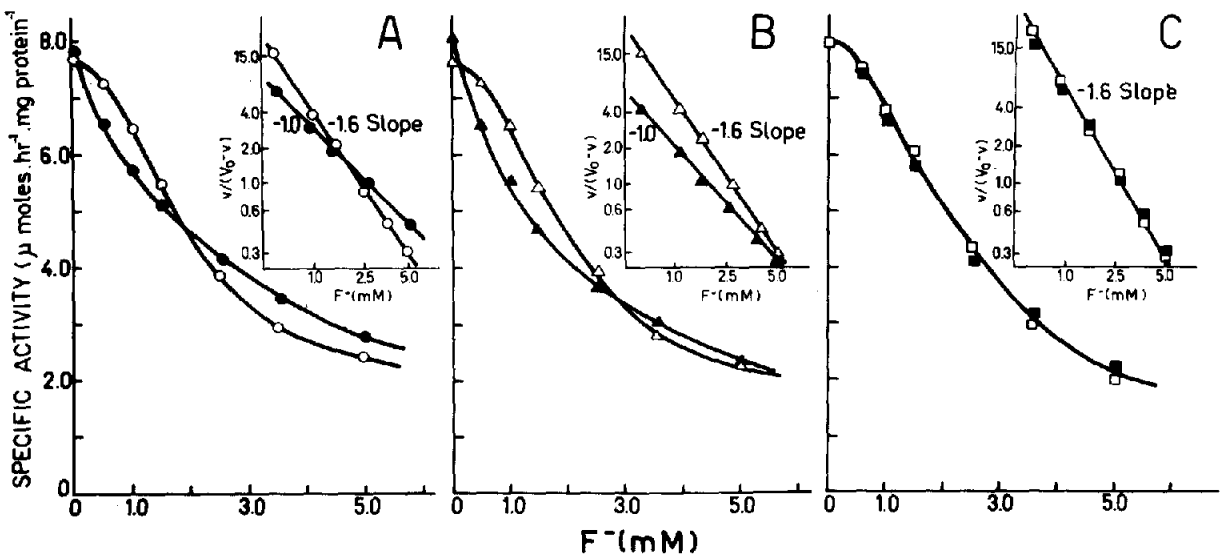

Fig.1. Effect of concentration of F^- on the reaction rate of membrane-bound acetylcholinesterase from rats fed a corn oil diet. (A) In the absence (○–○) and in the presence (●–●) of T_3 1×10^{-9} M. (B) In the presence of T_3 1×10^{-9} M and T_4 1×10^{-7} M (△ – △) and in the presence of T_3 1×10^{-9} M and T_4 1×10^{-8} M (▲ – ▲). (C) In the presence of T_4 1×10^{-7} M (□ – □) or T_4 1×10^{-4} M (■ – ■). Insets show Hill plots of same data. The same membrane preparation was used for control and hormone tests.

Table 1
Effect of T_3 and T_4 on *n* values of membrane-bound enzymes[a]

Hormone added (M)	Rat erythrocytes			*E. coli* growth at	
	Acetylcholinesterase		$(Na^+ + K^+)$ATPase	(Ca^{2+})ATPase	
	Corn oil diet	Lard diet	Corn oil diet	20°C	37°C
None	1.53 ± 0.02	1.00 ± 0.01	2.00 ± 0.04	2.30 ± 0.05	1.80 ± 0.03
T_3 1×10^{-9}	0.90 ± 0.05	0.95 ± 0.04	3.60 ± 0.24	1.20 ± 0.06	1.20 ± 0.04
T_4 1×10^{-7}	1.54 ± 0.04	0.95 ± 0.05		2.33 ± 0.10	1.80 ± 0.10
T_4 1×10^{-4}	1.55 ± 0.06	1.00 ± 0.03		2.20 ± 0.10	
T_3 1×10^{-9} + T_4 1×10^{-8}	1.00 ± 0.04			1.30 ± 0.10	1.15 ± 0.05
T_3 1×10^{-9} + T_4 1×10^{-7}	1.60 ± 0.01	1.05 ± 0.05	2.00 ± 0.02	2.20 ± 0.10	1.80 ± 0.05

[a] Mean of *n* values of 3–5 different enzymatic preparations ± SEM; values followed by different letters were significantly different ($p < 0.001$) when compared by the Student's *t*-test for paired samples

both T_3 1×10^{-9} M and T_4 1×10^{-7} M. These hormonal concentrations are within the strict order of mammalian physiological ranges [13,14]. When the concentration of T_4 was decreased from 1×10^{-7} M to 1×10^{-8} M, this hormone lost its blocking ability. When alone, T_4 1×10^{-7} M or 1×10^{-4} M does not have any effect on values of *n* of corn oil-fed rats (fig.1C). Table 1 summarizes these results.

3.1.2. Lard-fed rats

In the case of the rats grown on lard-supplemented diet, values close to 1.0 were found in all the assayed conditions (table 1). These facts ruled out the possibility that the blocking effect of T_4 on T_3 action could be due to an increment of values of *n* by T_4 action. The fact that there was no effect of T_3 on membrane from rats fed a lard diet does not necessarily imply that T_3 is unable to modify the fluidity of these systems, since in the low fluidity membrane the cooperative transition of the acetylcholinesterase is at its minimal expression and by this probe no additional changes in membrane fluidity can be evaluated. This theoretical point has been discussed [4,5].

Neither the specific activity (in the absence of F^-) nor the $K_{0.5}$ of the enzyme from both diets were modified by the presence of T_3 or T_4 or by different combinations of them.

3.2. *Effect of T_3 on soluble acetylcholinesterase*

A Hill coefficient of 1.6 has been reported for solubilized acetylcholinesterase from rat membrane erythrocyte irrespective of the kind of dietary lipid used [1,7,12,15]. As found with insulin and cortisol [16], values of 1.6 were also obtained in the soluble form of the enzyme in the presence of T_3 1×10^{-9} M (not shown). That is, T_3 exerted its effect only when the acetylcholinesterase was bound to a structured membrane.

3.3. *Effect of T_3 and T_4 on the allosteric inhibition by F^- of membrane-bound $(Na^+ + K^+)$ATPase*

Whether the above effects were mediated by a change in bulk membrane fluidity rather than by a local change in fluidity near the acetylcholinesterase molecules, or by some other more direct hormonal interaction with the enzyme (i.e., by binding to the acetylcholinesterase), one could expect an opposite change in the values of *n* for the F^- inhibition of the $(Na^+ + K^+)$ATPase. This enzyme showed an inverse correlation (r –0.82) between Hill coefficient and membrane fatty acid fluidity [1]. As it can be observed in table 1, T_3 caused a significant increment in the *n* values of the $(Na^+ + K^+)$ATPase from rat fed a corn oil diet (from 2.1–3.6). The blocking action of T_4 is also present in this enzymatic system.

3.4. *Effects of T_3 and T_4 on allosteric behavior of membrane (Ca^{2+})ATPase from* Escherichia coli

It was shown that the allosteric inhibition by Na^+ of membrane-bound (Ca^{2+})ATPase from *E. coli* was dependent upon the fatty acid composition of the cell membrane [17]. High positive correlations (r 0.94) were obtained between the values of *n* and

membrane fluidity [3,18]. Table 1 shows the T_3 and T_4 actions and their interplay on the values of n determined at 36°C from *E. coli* grown at either 20°C or 37°C. The presence of T_3 produced a decrease in the values of n in both high or low membrane fatty acid fluidities. Under the influence of T_4 alone, there is no effect on the membrane enzyme but, as in the case of the erythrocyte membrane system, it blocked the T_3 action. The allosteric behavior of the soluble (Ca^{2+})ATPase was not affected by the presence of the T_3 (not shown).

4. Discussion

4.1. *Effect of T_3 on membrane fluidity*

The facts that:

(i) T_3 action was only observed in membrane-bound enzymes;

(ii) T_3 decreased the n values of erythrocyte acetylcholinesterase and *E. coli* (Ca^{2+})ATPase and enhanced it in the erythrocyte $(Na^+ + K^+)$ATPase system;

(iii) The correlations between the membrane fluidity and the values of n for the former enzymes were positive [1,3] whereas for the latter enzyme it was negative [1];

constitute strong evidence for the hypothesis that T_3 decreases the membrane fluidity. Half-maximal effects on values of n of acetylcholinesterase were obtained at 6×10^{-11} M (about 0.03 ng/ml) [6] of T_3, which is one-hundred times lower than its physiological concentration [13,14].

A binding site for T_3 of high affinity and limited capacity in rat erythrocyte membrane was found (unpublished results). It was reported that rabbit erythrocyte membranes bind both T_4 and T_3 [19].

4.2. *Blocking effect of T_4 on the T_3 action*

Since it was shown that T_3 is biologically more potent than T_4 [20,21] and that there is an extra-thyroidal conversion from T_4 to T_3 [22–24], there has been speculation as to whether T_3 is the only biologically active thyroid hormone and that T_4 might be a more prohormone for T_3 [23,25,26]. However, several workers suggested that T_4 could have a true biological activity per se [27–29]. Some membrane systems were affected by thyroid hormones [30–33]; in our case, T_3 decreased membrane fluidity. T_4 showed an antagonistic effect on the T_3 action. This blocking effect of T_4 on T_3 action is unusual, since the biological potence of T_4 is similar or lower than T_3, but both hormones act in the same direction [19–21, 27–29]. This finding represents, to the best of our knowledge, the first observation of an hormonal blocking action of the T_4 on T_3.

The studies with thyroid hormone analogues indicated a very high molecular specificity for L-T_3 and L-T_4 actions. L-Alanine side chain is essential to carry out both allosteric desensitization and blocking effects. The blocking ability is characterized by the presence of iodine in position 5′ (unpublished results).

4.3. *Membrane cooperative enzymes and thyroid hormone actions*

Several features of the changes in the cooperative behavior of membrane enzymes in the erythrocyte membrane systems distinguish them from the corresponding changes in bacterial systems, e.g., the effectors are F^- and Na^+, respectively, and enzymes and membranes differ in several aspects. The acetylcholinesterase and $(Na^+ + K^+)$ATPase from rat erythrocytes and (Ca^{2+})ATPase from *E. coli* have different localization in the membrane, they have different dependence on the lipids for their enzymatic activity, and differ also in their metabolic function [4]. The membranes from rat erythrocytes and *E. coli* differ largely in properties, functions and composition, e.g., the bacterial membrane does not contain sterol whereas the erythrocyte membrane has one of the highest cholesterol/phospholipid ratios [34]. However, all the facts observed in rat erythrocyte and *E. coli* systems appear as a response to the general regulatory property of the T_3 and T_4 interplay on the membrane cooperative enzymes through changes in the membrane fluidity. Thus, this interdependence is not confined to one system. This suggestion may constitute a challenge in thyroid research field.

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