Stimulation of calcium-ATPase activity by 3,5,3'-tri-iodothyronine in rat thymocyte plasma membranes

A possible role in the modulation of cellular calcium concentration

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We have previously demonstrated that 3,5,3'-tri-iodo-L-thyronine (T_3) produces a very rapid and transient increase in calcium uptake and cytoplasmic free calcium concentration in the rat thymocyte, and have postulated that Ca^{2+} -ATPase may contribute to the overall effect of T_3 on cellular calcium metabolism. In the present study, we show that in the rat thymocyte, T_3 increased plasma membrane Ca^{2+} -ATPase activity. This effect of T_3 was very rapid, seen at 30 s after the addition of the hormone, and was concentration-related, evident at a physiological concentration as low as 1 pM. Evaluation of the effect of several thyronine analogues on Ca^{2+} -ATPase activity revealed the following order of potency: $D-T_3 \geq 3'$ -isopropyl-L- $T_2 = L-T_3 = L-T_4 = D-T_4 > L-rT_3 > 3,5$ -L- $T_2 > DL$ -thyronine. Studies with the calmodulin antagonist trifluoperazine demonstrated that thymocyte Ca^{2+} -ATPase activity and its stimulation by T_3 are influenced by calmodulin. Other studies showed that several adrenergic agents, agonists and antagonists, had no effect on thymocyte Ca^{2+} -ATPase activity and its stimulation by T_3 . From these and previous observations, we would suggest that in the rat thymocyte, the T_3 -induced increase in Ca^{2+} -ATPase activity, which enhances the expulsion of calcium from the cell, plays a role in the diminution and transiency of the stimulatory effect of T_3 on thymocyte calcium metabolism.

INTRODUCTION

In the rat thymocyte, 3,5,3'-tri-iodothyronine (T_3) acts at the level of the plasma membrane to increase adenylate cyclase activity, cellular cyclic AMP concentration and the uptake of the glucose analogue 2-deoxyglucose (2-DG). All these effects of T_3 require calcium (Segal & Ingbar, 1979, 1981; Segal *et al.*, 1985). We have recently demonstrated in the rat thymocyte that T_3 produces a very rapid, almost immediate, increase in extracellular calcium uptake which is followed by an increase in cytoplasmic free calcium concentration. This is the most rapid effect of T_3 in this and other systems (Segal & Ingbar, 1984, 1985; Segal, 1988). From these and other observations we have postulated that calcium [which exerts its action by activating calmodulin which, in turn, acts to increase adenylate cyclase activity (Segal *et al.*, 1986)] serves as the first messenger for the plasma-membrane-mediated action of T_3 in the rat thymocyte (Segal *et al.* 1985; Segal, 1988).

Evaluation of the effect of T_3 on thymocyte ^{45}Ca uptake and cytoplasmic free calcium concentration, in the presence or absence of a physiological concentration of extracellular calcium (1 mM), suggests that the regulation of thymocyte calcium metabolism by the thyroid hormone is associated with the activation of the plasma membrane Ca^{2+} -ATPase, a key regulator of cellular

calcium concentration. This contributes to the transiency of the effect of T_3 on ^{45}Ca uptake and cytoplasmic free calcium concentration in the rat thymocyte (Segal, 1988). Support for this hypothesis comes from the studies of Davis and co-workers (Davis & Blas, 1981; Rudinger *et al.*, 1984) who have demonstrated in the human erythrocyte and the rabbit heart that T_3 increases Ca^{2+} -ATPase activity.

To test this possibility, we examined the effect of T_3 on Ca^{2+} -ATPase activity in isolated rat thymocyte plasma membranes. We demonstrate here that T_3 increased thymocyte Ca^{2+} -ATPase activity, an effect that was prompt in onset, concentration-related and evident at a physiological concentration of 1 pM- T_3 .

EXPERIMENTAL

Animals used in this study were 25–28-day-old female rats of the Sprague–Dawley strain [CrI:CD (SD) BR; Charles River Laboratories, Wilmington, MA, U.S.A.]. [These animals were maintained in accordance with the guidelines of the Committee on Animals of the Harvard Medical School and those prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, National Research Council (DHED publication No. (NIH) 78-23, revised

Abbreviations used: T_3 , 3,5,3'-tri-iodo-L-thyronine; T_4 , 3,5,3',5'-tetra-iodo-L-thyronine; $D-T_3$, 3,5,3'-tri-iodo-D-thyronine; $D-T_4$, 3,5,3',5'-tetra-iodo-D-thyronine; rT_3 , 3,3',5'-tri-iodo-L-thyronine; 3'-isopropyl- T_2 , 3'-isopropyl-3,5-di-iodo-L-thyronine; 3,5- T_2 , 3,5-di-iodo-L-thyronine; 2-DG, 2-deoxyglucose.

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1978).] Thymocytes were isolated and plasma membranes were prepared according to a procedure described previously (Segal *et al.*, 1985).

Materials

Materials used in this work were purchased from the following sources: EGTA, ouabain, Tris-ATP, DL-thyronine and Tris/HCl from Sigma Chemical Co., St. Louis, MO, U.S.A.; T_3 , D- T_3 , T_4 , D- T_4 , and 3,5- T_2 from Henning Berlin, Germany; r T_3 from Calbiochem-Behring, San Diego, CA, U.S.A. 3'-isopropyl- T_2 was a gift from Henning.

Ca²⁺-ATPase

Membranes were suspended in the reaction buffer which consisted of 110 mM-KCl/20 mM-NaCl/50 mM-Tris/HCl, pH 7.4. This 'Ca²⁺- and Mg²⁺-free' medium contains 2 μ M-Ca²⁺ and 1.5 μ M-Mg²⁺ (measured by atomic absorption flame photometry; Perkin-Elmer spectrophotometer model 303) as contaminants of the buffer salts. Aliquots of 50 μ g of membrane protein were transferred into small plastic tubes (reaction medium, 500 μ l final volume) containing 0.2 mM-ouabain, and CaCl₂, MgCl₂, and EGTA in various concentrations and combinations. Membranes were allowed to equilibrate in a shaking water bath for 10 min at 37 °C in air, and the enzymic reaction was begun by addition of 5 mM-Tris-ATP. The reaction was stopped by the addition of 500 μ l of ice-cold 12 % trichloroacetic acid. Samples were allowed to stay in ice for 20 min, and were then centrifuged at 1500 *g* for 20 min. The supernatant was collected, and its content of P_i was measured by the phosphomolybdate procedure described by Ames (1966). Ca²⁺-ATPase activity was determined by subtracting P_i values obtained in the Ca²⁺-free medium (with 0.1 mM-EGTA) from those obtained in the presence of calcium. Parallel specimens were kept in ice throughout the entire incubation period to assess values for the non-enzymic hydrolysis of ATP. Non-enzymic hydrolysis was the same in the presence or absence of membranes and was not changed by T_3 .

The isolated plasma membranes contained a substantial amount of calcium (20 nmol/mg of protein) and magnesium (800 nmol/mg) on trichloroacetic acid extraction (measured by flame spectrophotometry using a Perkin-Elmer spectrophotometer, model 303). The majority of these two bivalent cations is tightly bound to the plasma membrane, and following an incubation at 37 °C for 60 min only a fraction is released, i.e. 25 % and 2.5 % of calcium and magnesium respectively, of which about 90 % is released during the first 15 min. Thus, the Ca²⁺ concentration in the 500 μ l of reaction medium incubated with 50 μ g of plasma-membrane protein is enriched by 0.5 μ M-calcium and 2 μ M-magnesium. In a medium devoid of added CaCl₂ (Ca²⁺ concentration of 2.5 μ M), and with or without added 3 μ M-MgCl₂ (Mg²⁺ concentration of 3.5 or 6.5 μ M respectively), Ca²⁺-ATPase activity was essentially nil. A consistent enzymic activity became apparent only at about 10 μ M-calcium. This demonstrates that the so-called calcium-high-affinity enzymic activity is not evident in this preparation of rat thymocyte plasma membranes. At various Mg²⁺ concentrations, and in the absence of added calcium (Ca²⁺-free medium), ATP hydrolysis was the same in the presence or absence of 0.1 mM-EGTA [EGTA binding of Mg²⁺ is small and essentially does not change free Mg²⁺

Table 1. The effect of calcium and magnesium on Ca²⁺-ATPase activity in rat thymocyte plasma membranes

Membranes were equilibrated for 10 min with calcium, magnesium and EGTA in various concentrations and combinations. Then ATP was added and the reaction was stopped 30 min later. Ca²⁺-ATPase activity was calculated by subtracting P_i values produced in the absence of calcium from those obtained in the presence of calcium, in the respective magnesium group. Values shown are the means $\pm$ S.D. obtained from three separate experiments.

MgCl ₂ (μ M)	CaCl ₂ (1 mM)	EGTA (0.1 mM)	Total ATPase activity (μ mol of P _i / mg of protein)	Ca ²⁺ -ATPase activity (μ mol of P _i / mg of protein)
3	—	—	0.0 $\pm$ 0.0	
3	+	—	1.3 $\pm$ 1.1	1.3 $\pm$ 1.1
3	—	+	0.0 $\pm$ 0.0	
50	—	—	0.2 $\pm$ 0.3	
50	+	—	4.2 $\pm$ 1.5	4.1 $\pm$ 1.5
50	—	+	0.1 $\pm$ 0.1	
500	—	—	2.3 $\pm$ 2.0	
500	+	—	4.2 $\pm$ 1.7	2.1 $\pm$ 0.5
500	—	+	2.1 $\pm$ 1.6	
5000	—	—	4.3 $\pm$ 1.3	
5000	+	—	3.5 $\pm$ 1.0	-0.8 $\pm$ 0.7
5000	—	+	4.1 $\pm$ 1.1	

concentration; the Mg-EGTA association constant (K_a) at 37 °C is about 10² M⁻¹ (Pershad Singh & McDonald, 1980; Lin *et al.*, 1983) (Table 1)]. Interestingly, in this situation, i.e. presence of Mg²⁺ and absence of Ca²⁺, T_3 had no effect on Ca²⁺-ATPase activity. Note that, by definition, the enzyme involved is Mg²⁺-ATPase. However, because in the present preparation Mg²⁺ can replace Ca²⁺ in promoting ATP hydrolysis, it is not possible to determine whether here ATP hydrolysis is processed through two different enzymes or through a single enzyme.

Measurement of enzyme activity in the presence of Ca²⁺ (10–5000 μ M) and Mg²⁺ in various concentrations and combinations revealed (1) that Ca²⁺-ATPase became more prominent in the presence of Mg²⁺ (Mg²⁺ was also essential for a reproducible activity of Ca²⁺-ATPase), (2) that optimal Ca²⁺-ATPase activity was reached at 50 μ M-MgCl₂, and (3) that Mg²⁺ at high concentrations can replace Ca²⁺ in activating Ca²⁺-ATPase (Table 1; results shown only with 1 mM-Ca²⁺).

In the ensuing experiments, the medium for measurement of enzymic activity contained 50 μ M-MgCl₂. Because in the Ca²⁺-free medium, values of ATP hydrolysis were the same in the presence and absence of 0.1 mM-EGTA (values of the former are subtracted from enzyme activity in the presence of Ca²⁺), ATP hydrolysis measured is that of Ca²⁺-influenced ATPase activity devoid of Mg²⁺-ATPase activity. Since such activity is better expressed in the presence of Mg²⁺, the ATP hydrolysis measured here is that of Ca²⁺(Mg²⁺)-ATPase, which hereinafter is called Ca²⁺-ATPase.

Protein concentration

Protein concentration was measured according to a method described by Lowry and co-workers (1951).

Fig. 1. Effect of calcium concentration on Ca²⁺-ATPase activity in rat thymocyte plasma membranes

Membranes were incubated with various concentrations of calcium for 30 min and their Ca²⁺-ATPase activity was measured. Calcium concentration shown is that of CaCl₂ added to the medium. Results shown are from a single experiment and are typical of those obtained in two other different experiments.

Plasma membrane protein concentration was measured prior to experimentation, and 50 μg of plasma membrane protein was then transferred to the assay tubes.

Statistical analysis

Where appropriate, the Dunnett's test was used for comparisons between experimental groups and a single control, and the analysis of variance followed by the Newman-Keuls multiple range test for comparisons of multiple experimental groups with each other (Zar, 1974).

RESULTS

Calcium concentration

Plasma membranes were incubated for 30 min with various concentrations of calcium, ranging from 2.5 μM ('Ca²⁺-free' medium) to 5 mM in the presence of 50 μM-MgCl₂, and their Ca²⁺-ATPase activity was measured. [Note that Ca²⁺ concentrations shown are those of CaCl₂ added to the reaction medium and not free Ca²⁺ concentration. In the isolated plasma membrane system, the effective free Ca²⁺ concentration cannot be determined accurately, primarily because of a relatively large, unknown calcium-binding capacity of different membrane components (Segal, 1986).]

Enzyme activity increased progressively with the increase in calcium concentration, reaching maximal activity between 1 and 5 mM-Ca²⁺ (Fig. 1); 50% of maximum activity was achieved at about 0.3 mM-Ca²⁺.

At 5 mM-Ca²⁺, only about 10% of total ATP added was hydrolysed, indicating that at this Ca²⁺ concentration substrate availability was not a limiting factor and that indeed enzyme activity had reached maximal values.

Time response

After an equilibration period of 10 min, ATP was added to membranes suspended in medium containing 0.3 or 1 mM-Ca²⁺, and enzyme activity was measured at various points in time thereafter. Enzyme activity was sluggish during the first 5 min of incubation, more so at the lower concentration of calcium, and then increased

Fig. 2. Ca²⁺-ATPase activity in rat thymocyte plasma membranes: time course

Membranes were incubated with 0.3 (●) or 1.0 (○) mM-Ca²⁺ and their Ca²⁺-ATPase activity was measured after various periods of time. Values shown are the mean ± S.D. obtained from three separate experiments.

Fig. 3. Effect of T₃ on Ca²⁺-ATPase activity in rat thymocyte plasma membranes

Membranes were equilibrated in a reaction medium containing 150 μM-Ca²⁺, 100 μM-EGTA, and 50 μM-Mg²⁺ (a concentration at which maximal response to T₃ was observed) at 37 °C for 10 min. Then ATP was added and 20 min later T₃ in various concentrations was added. The reaction was stopped 0.5, 10 or 30 min thereafter. Values shown are the means ± S.E.M. obtained from four separate experiments. * (*P* < 0.05) and ** (*P* < 0.01), significantly greater than the corresponding control.

Fig. 4. Effect of several thyronine analogues on Ca^{2+} -ATPase activity in rat thymocyte plasma membranes

The experimental procedure was the same as that described in the legend to Fig. 3, except that incubation with the thyronine analogues was for 30 s. Values shown are the means (S.D. values varied from 0.04 to 0.19) obtained from two (DL-thyronine), four (3'-isopropyl- T_2 , rT_3 and 3,5-L- T_2), or five (L- T_3 , L- T_4 , D- T_3 and D- T_4) separate experiments. A half-maximal effect was observed at a concentration of 1 pM for D- T_3 , and 4 pM for L- T_3 , 3'-isopropyl- T_2 , L- T_4 and D- T_4 .

linearly with incubation time; that is, from 5 to 60 min of incubation, enzyme activity (ATP hydrolysis per unit of time) remained about the same (Fig. 2).

In the ensuing experiments with T_3 (and other agents), following a 10 min equilibration period, membranes were first incubated with ATP for 20 min and then T_3 (or other agents) was added and its effect on enzyme activity was measured.

Effect of T_3

T_3 was added at various concentrations and its effect on Ca^{2+} -ATPase activity was measured after 0.5, 10 and 30 min. The maximal response to T_3 was evident in the presence of 150 μM - Ca^{2+} , 100 μM -EGTA and 50 μM - Mg^{2+} . T_3 produced a rapid and concentration-related increase in Ca^{2+} -ATPase activity. The effect of T_3 was already evident within 0.5 min, and about the same proportional increase in enzyme activity was seen also after 10 and 30 min of incubation (Fig. 3). The lowest effective concentration of T_3 was 1 pM at 30 min of incubation, and 10 pM after 0.5 and 10 min of incubation. Since the effect of T_3 on thymocyte calcium metabolism is very rapid, seen within 15–30 s, the influence of thyroid hormones on enzyme activity was measured for 30 s in the following experiments.

Thyronine analogues

Membranes were incubated for 30 s with various thyronine analogues at various concentrations, and their

Fig. 5. Inhibition by trifluoperazine of Ca^{2+} -ATPase activity in rat thymocyte plasma membranes

Membranes, suspended in medium containing 150 μM - Ca^{2+} , 100 μM -EGTA and 50 μM - Mg^{2+} , were first incubated for 10 min with various concentrations of trifluoperazine, then the enzymic reaction was started upon the addition of ATP and was stopped 30 min later. Results shown are the means obtained from two separate experiments.

effect on Ca^{2+} -ATPase activity was measured. The order of potency of the analogues was as follows: D- $\text{T}_3 \geq 3'$ -isopropyl- $\text{T}_2 = \text{L-T}_3 = \text{L-T}_4 = \text{D-T}_4 > \text{L-rT}_3 > 3,5\text{-L-T}_2 > \text{DL-thyronine}$ (Fig. 4). 50% of maximal activity was seen at 1 pM of D- T_3 and 4 pM of L- T_3 .

Noteworthy is the observation that the order of potency of thyronine analogues in stimulating Ca^{2+} -ATPase activity is different from that described for the other metabolic functions of thyroid hormones in the rat thymocyte, i.e. ^{45}Ca uptake, cytoplasmic free calcium concentration, adenylate cyclase activity, cyclic AMP concentration, and 2-DG uptake (Segal & Ingbar, 1979, 1981, 1984; Segal *et al.*, 1985).

Calmodulin

Since calmodulin modulates the Ca^{2+} -dependent stimulatory effects of T_3 in rat thymocytes (Segal & Ingbar, 1979, 1981; Segal *et al.*, 1985, 1986), and calmodulin also regulates the T_3 -induced increase in Ca^{2+} -ATPase activity in human erythrocyte ghosts and rabbit heart membranes (Davis *et al.*, 1983; Rudinger *et al.*, 1984), we have examined whether thymocyte Ca^{2+} -ATPase activity and its stimulation by T_3 are influenced by calmodulin.

Trifluoperazine, a calmodulin inhibitor (Levin & Weiss, 1977; Weiss, 1983; Segal *et al.*, 1986), produced a concentration-related decrease in Ca^{2+} -ATPase activity (and also in Mg^{2+} -ATPase activity; results not shown); 50% inhibition was achieved at 50 μM -trifluoperazine (Fig. 5). Trifluoperazine also blocked the stimulatory effect of T_3 on Ca^{2+} -ATPase activity (Fig. 6), and these inhibitory effects were abolished by the addition of calmodulin (Fig. 6). This indicates that trifluoperazine

Fig. 6. Effect of calmodulin on Ca²⁺-ATPase activity in rat thymocyte plasma membranes

Membranes were first incubated with or without trifluoperazine (TFP) and calmodulin (CaM) for 10 min. ATP was then added, and 20 min later T₃ was added and the reaction was stopped 30 s thereafter. Values shown are the means $\pm$ S.E.M. obtained from five separate experiments. * ($P < 0.05$) and ** ($P < 0.01$), significantly greater than the corresponding T₃-free and CaM-free group. † ($P < 0.05$) and †† ($P < 0.01$), significantly different from control T₃-free, CaM-free or TFP-free group.

exerted its inhibitory effect by virtue of its being a calmodulin inhibitor.

Catecholamines

Catecholamines have been shown to influence rat thymocyte metabolic functions and the stimulatory effect of T₃ thereupon; thus, adrenaline increases while alprenolol blocks the action of T₃ (Segal & Ingbar, 1980, 1981; Segal *et al.*, 1985). Here, we examined the effect of various catecholamines (agonists and antagonists), in concentrations ranging from 0.1 μ M to 10 μ M [concentrations which influence metabolic functions in the rat thymocyte (Segal & Ingbar, 1980, 1981; Segal *et al.*, 1985)], on thymocyte Ca²⁺-ATPase activity and its stimulation by T₃. The agonists used were adrenaline, noradrenaline, isoproterenol, prenalterol, Zinterol and phenylephrine, and the antagonists used were alprenolol, propranolol and phentolamine. None of these adrenergic agents changed Ca²⁺-ATPase activity or its stimulation by T₃ (results not shown).

DISCUSSION

Observations in rat thymocytes show that calcium has a central role in, and possibly is the first messenger for, the plasma membrane-mediated action of T₃ in promoting adenylate cyclase activity, cellular cyclic AMP concentration and sugar uptake (Segal & Ingbar, 1981; Segal *et al.*, 1985; Segal, 1988). Furthermore, our studies of the effect of T₃ on thymocyte ⁴⁵calcium uptake and cytoplasmic free calcium concentration point to a possible role for Ca²⁺-ATPase there (Segal, 1988).

In the present study, we demonstrate that indeed T₃ increased the plasma membrane Ca²⁺-ATPase activity in the rat thymocyte. This stimulatory effect of T₃ was very prompt in onset, being evident within 30 s of the addition of the hormone. It was concentration-related, the lowest effective concentration being 1 pM, and was influenced by calmodulin. These properties of the hormonal effect are in accord with the observations of Davis and co-workers in human erythrocyte ghosts and rabbit-heart plasma membranes (Davis & Blas, 1981; Davis *et al.*, 1983; Rudinger *et al.*, 1984).

In the rat thymocyte, unlike several other tissues such as liver, white fat and corpus luteum (Pershad Singh & McDonald, 1980; Lotersztajn *et al.*, 1981; Verma & Penniston, 1981), plasma membranes were devoid of the so-called high-affinity Ca²⁺-ATPase activity (enzyme activity which is saturated at about 1 μ M-Ca²⁺), but demonstrated a consistent enzyme activity only in the presence of calcium (and magnesium) at a concentration of 10 μ M or greater. This indicates that, as in the intact cell, in isolated thymocyte plasma membranes, which are arranged in closed, perforated and ATP-permeable (Hochstadt *et al.*, 1975) vesicles primarily (over 90%) at the right-side-out orientation (Ferber *et al.*, 1972; Schmidt-Ullrich *et al.*, 1974; Segal & Ingbar, 1982), Ca²⁺-ATPase activity responds to and is activated by an increase in intravesicular calcium concentration above a given value.

The present study together with previous observations (Segal & Ingbar, 1984; Segal, 1988) on the effect of T₃ on thymocyte ⁴⁵Ca uptake and cytoplasmic free calcium concentration [in the presence or absence of physiological concentration of extracellular calcium (1 mM)], supports our hypothesis that the plasma membrane Ca²⁺-ATPase contributes to the gradual decline and transiency of the calcium-dependent plasma membrane-mediated effects of T₃.

Thus, as proposed in the heart and in several other tissues (Langer, 1976, 1980; Berridge, 1984), in the rat thymocyte, in the presence of physiological concentration of extracellular calcium, the initial increase in calcium uptake and thereby in cytoplasmic calcium concentration triggers the release of calcium from intracellular pool(s) (most likely the endoplasmic reticulum) resulting in a further increase in the cytoplasmic free calcium concentration. This, in turn, acts to increase various Ca²⁺-dependent metabolic functions in the cell; one of which is the Ca²⁺-ATPase which acts to maintain cytoplasmic calcium concentration at a low, physiologically functioning level (Roufogalis, 1979; Pershad Singh & McDonald, 1980; Verma & Penniston, 1981). The increase in Ca²⁺-ATPase activity results in an increase in calcium exit from the cell and thereby contributes to the gradual decline in the Ca²⁺-dependent effects of T₃.

It should be noted, however, that in the rat thymocyte the effect of T₃ on the plasma membrane Ca²⁺-ATPase activity described here differs in several properties from the previously described effects of T₃ on other metabolic functions such as calcium uptake and cytoplasmic free calcium concentration, adenylate cyclase activity, cellular cyclic AMP concentration and sugar uptake (Segal & Ingbar, 1979, 1981, 1985; Segal *et al.*, 1985; Segal, 1988). These differences include: (1) a greater sensitivity of Ca²⁺-ATPase to T₃, 1 pM (Ca²⁺-ATPase) versus 1 nM (others) being the lowest effective concentration; (2) a different order of potency for the thyroid hormone

analogues: for activation of Ca^{2+} -ATPase, the L- and D-isomers of T_3 and T_4 are equally potent; for the other metabolic functions, L- T_3 is more effective than L- T_4 and the L-isomers are more potent than their D-counterparts; and (3) adrenergic agents (agonists and antagonists) which influence the other metabolic functions and their stimulation by T_3 , had no effect on Ca^{2+} -ATPase activity and its stimulation by T_3 .

At present, we are unable to explain these differences, unless they relate to two separate and independent systems. Rather, we would suggest that, in the rat thymocyte, plasma membrane Ca^{2+} -ATPase activity is associated with the overall metabolic activities of T_3 at the level of the plasma membrane, and that the T_3 -induced increase in calcium uptake and cytoplasmic free calcium concentration is accompanied by an increase in Ca^{2+} -ATPase activity, which results in an increase in the extrusion of calcium from the cell and in the diminution, and thereby transiency, of the effects of T_3 on thymocyte calcium metabolism, adenylate cyclase activity, cyclic AMP concentration and 2-DG uptake.

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