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Studies of the mechanism by which 3,5,3'- triiodothyronine stimulates 2-deoxyglucose uptake in rat thymocytes in vitro. Role of calcium and adenosine 3':5'-monophosphate.

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Research Article

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Studies of the Mechanism by Which 3,5,3′-Triiodothyronine Stimulates 2-Deoxyglucose Uptake in Rat Thymocytes In Vitro

ROLE OF CALCIUM AND ADENOSINE 3′:5′-MONOPHOSPHATE

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ABSTRACT The present experiments were designed to explore the mechanism whereby 3,5,3′-triiodothyronine (T_3) stimulates the uptake of 2-deoxy-D-glucose (2-DG) in rat thymocytes in vitro. Addition of T_3 evoked a transient, dose-related increase in cellular cyclic (c) AMP concentrations, evident within 5 min, followed soon by an increase in 2-DG uptake. The effects of T_3 on both cAMP concentration and 2-DG uptake were dependent upon the presence of extracellular calcium. Epinephrine also induced a sequential increase in thymocyte cAMP concentration and 2-DG uptake. These responses were more prompt than those to T_3, but were calcium independent. As with their combined effects on 2-DG uptake, T_3 and epinephrine produced synergistic or additive effects on cellular cAMP concentration. Dibutyryl cAMP also stimulated 2-DG uptake, an effect that was more prompt than that of epinephrine, and, like that of epinephrine, was calcium independent.

Prior or simultaneous addition of L-alprenolol (10 μM), which, we have previously shown, blocks the effect of both T_3 and epinephrine on 2-DG uptake, also blocked the increase in thymocyte cAMP concentration induced by these agents. In contrast, L-alprenolol failed to block the increase in 2-DG uptake produced by dibutyryl cAMP.

On the basis of these observations we suggest that T_3 increases 2-DG uptake in the rat thymocyte by increasing the cellular concentration of cAMP, which then acts to enhance sugar transport. The increase in 2-DG uptake induced by epinephrine is also mediated by an increase in cAMP concentration. The greater response of cellular cAMP concentration to T_3 and epinephrine when added together than to either agent added alone may explain their synergistic action to increase 2-DG uptake. We suggest that these actions of T_3 and epinephrine are both initiated at the level of the plasma membrane.

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INTRODUCTION

In previous studies, we have demonstrated that 3,5,3′-triiodothyronine (T_3)[1] enhances the transport of the glucose analogues 2-deoxy-D-glucose (2-DG) and 3-*O*-methylglucose (3-*O*-MG) into rat thymocytes in vitro (1). This effect is prompt and independent of new protein synthesis. T_3 and epinephrine act synergistically to increase sugar uptake into the cell (2), and, in the presence of both epinephrine and insulin, 2-DG uptake is enhanced by a physiological (5 pM) concentration of T_3 (3).

Both calcium and cyclic (c) AMP have been implicated in the regulation of the uptake of sugar in various tissues (4–10). In addition, thyroid hormone has been demonstrated to affect both calcium (11–13) and cAMP (14–18) metabolism in different tissues. Further, in the rat thymocyte, T_3 acts synergistically with epinephrine to increase 2-DG uptake, and epinephrine is known to affect the metabolism of both calcium (19–21), and cAMP (22–25). These considerations led us to explore the role that calcium and cAMP might play in the stimulatory effect of T_3 on the sugar uptake of thymocytes. The results of these studies are the substance of this report.

[1] *Abbreviations used in this paper*: Bt_2cAMP, dibutyryl cyclic AMP; 2-DG, 2-deoxy-D-glucose; 3-*O*-MG, 3-*O*-methylglucose; T_3, L-3,5,3′-triiodothyronine.

METHODS

Preparation of thymocyte suspensions. Thymocytes were isolated from female rats, 25–29 d old, of the CD strain (Charles River Breeding Laboratories, Wilmington, Mass.) by a method that we have described previously (1). In short, animals were killed by cervical dislocation. Thymus glands were then quickly removed, washed in ice-cold Krebs-Ringer-25 mM Tris buffer, pH 7.4, and gently teased with forceps. The cells thereby freed were filtered through nylon mesh, collected in plastic tubes, and centrifuged at 300 *g* for 10 min. The supernate was then decanted and the cells were resuspended in the buffer to yield a final concentration of 45×10^6 cells/ml. Principally, two types of buffer were used: (*a*) standard Krebs-Ringer-25 mM Tris buffer, pH 7.4, which contains 1 mM calcium, and (*b*) calcium-free medium, which is identical except that no calcium salts are added. This buffer contains 5 μM calcium owing to contamination by calcium in the other buffer salts. The standard medium was routinely used unless otherwise stated. 3 ml of suspended thymocytes was transferred into siliconized 25-ml Erlenmeyer flasks and was equilibrated for 30 min at 37°C in air. Thereafter, the cells were preincubated or incubated with various agents under conditions described below.

Measurement of [^{3}H]2-DG uptake. Thymocytes were preincubated with and without various agents for differing periods. At the end of preincubation period, [^{3}H]2-DG (8.26 Ci/mmol sp act)[2] was added to the medium in a final concentration of 3 μCi/ml, and the incubation was continued for another 15 min. At the end of the incubation period, 220-μl aliquots were quickly removed into microtubes (Beckman Instruments, Palo Alto, Calif.) and centrifuged at 10,000 rpm (Beckman microfuge) for 30 s. Supernates were removed by aspiration, the cell pellets were transferred to counting vials containing Toluene-Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.) scintillation liquid, and their ^{3}H content was measured in a β-scintillation counter with automatic quench correction.

Measurement of cellular cAMP concentrations. At the end of the equilibration period, the agents to be tested were added to the medium and the incubations continued for various periods. Cells were then transferred to plastic tubes and centrifuged at 800 *g* for 2 min at 4°C. The supernate was discarded, the tubes were quickly put in ice, and a tracer quantity of [^{3}H]cAMP (0.01 μCi; 40.2 Ci/mmol sp act) was added to permit measurement of the recovery of cAMP. The cell pellet was then suspended in 1.2 ml of ice-cold 6% trichloroacetic acid. The suspension was placed on ice for 20 min and was then centrifuged at 1,700 rpm for 15 min at 4°C. Aliquots of the supernate (1.0 ml) were taken, and their content of cAMP was measured by radioimmunoassay (Becton-Dickinson radioimmunoassay). Aliquots of the trichloroacetic acid-soluble fraction were transferred to scintillation vials containing Toluene-Triton X-100, and their [^{3}H]cAMP content was counted. Generally, a recovery of 93–98% was obtained.

Correction for occluded extracellular fluid. The extracellular fluid occluded in the pellet was assessed by means of [^{3}H]mannitol as an extracellular marker, as previously described (1).

Cell viability. The viability of the cells was assessed using the trypan blue technique (26), in which cells are suspended in 0.3% trypan blue solution (in saline) and their viability defined by their ability to exclude the dye. Unless otherwise stated, cell viability in all experimental groups was not different from control values (90–95%).

Statistical analysis. Where appropriate, statistical differences between experimental groups were evaluated using Dunnet's test for comparisons between multiple samples and a single control, and analysis of variance followed by the Neuman-Keuls multiple range test for comparisons among multiple groups (27).

RESULTS

Effects of T_3

Effect on 2-DG uptake: calcium dependence. Initial studies examined the uptake of 2-DG by thymocytes incubated in standard medium containing Ca^{2+} at a physiological concentration (1 mM) and in medium lacking Ca^{2+} (calcium-free medium). Although basal 2-DG uptake was the same in both types of medium, the stimulatory effect of both 1 nM and 1 μM T_3 on 2-DG uptake evident in standard medium was not evident in calcium-free medium (Fig. 1).

Addition of 1 mM EGTA to calcium-free medium produced a significant (10–15%) depletion of calcium from cells that had been preloaded with ^{45}Ca (data not shown). Basal uptake of 2-DG by thymocytes incubated in calcium-free medium containing 1 mM EGTA was

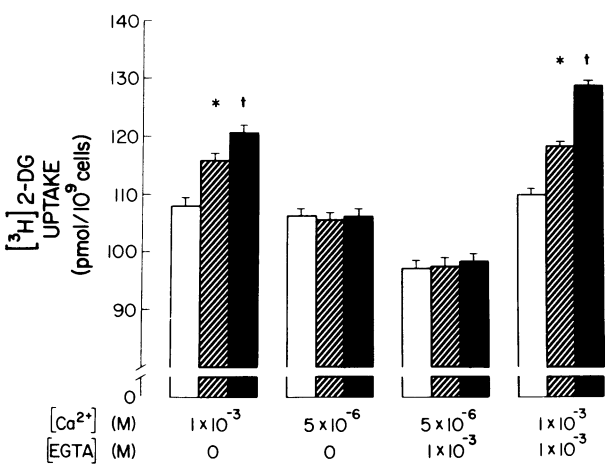

FIGURE 1 Calcium dependence of the effect of T_3 on the uptake of [^{3}H]2-DG by rat thymocytes. Cells were incubated in standard medium (1 mM Ca^{2+}) or in calcium-free medium (5 μM Ca^{2+}), with or without 1 mM EGTA. Thymocytes were then preincubated in the absence or presence of T_3, 1 nM (▨) or 1 μM (■), and 60 min later their 15 min uptake of [^{3}H]2-DG was measured as described in Methods. Values shown are mean±SD of those obtained in four separate experiments. *$P < 0.01$ vs. corresponding control group (□); †$P < 0.01$ vs. corresponding control and calcium-free groups.

[2] L-3,5,3′-Triiodothyronine was purchased from Henning Berlin GMBH (Berlin, Germany). EDTA, theophylline caffeine, L-epinephrine, L-isoproterenol, L-alprenolol, cAMP, and Bt_2cAMP were purchased from Sigma Chemical Co. (St. Louis, Mo.). D-alprendol was a gift from Hassle (Sweden). cAMP radioimmunoassay kit was purchased from Becton Dickinson Immunodiagnostic (Orangeburg, N. Y.). D-[2-Deoxy-^{3}H]glucose was purchased from New England Nuclear (Boston, Mass.).

decreased by ~10% ($P < 0.01$) from values obtained in standard medium, and, as in calcium-free medium alone, the response to T_3 was abolished. Addition of 1 mM Ca^{2+} to the calcium-free medium containing 1 mM EGTA restored both the basal uptake of 2-DG and the stimulatory response to T_3.

Like the effect of T_3 on 2-DG uptake when added alone, the synergistic interaction of T_3, both with epinephrine and with epinephrine plus insulin, was Ca^{2+} dependent (data not shown).

Effect on thymocyte cAMP concentration. It has been suggested that cAMP mediates the increase in sugar uptake induced by various agents in several tissue systems (8–10). Therefore, we examined the effect of T_3 on cAMP concentrations in the rat thymocyte. Initial studies examined the cAMP concentration in thymocytes at varying intervals after the addition of a high concentration of T_3 (10 μM). No change in the concentration of cAMP was evident 2 min after the addition of T_3, but by 5 min a highly significant increase in cAMP concentration (28±2.4%; mean±SD: $P < 0.01$) was seen (Fig. 2). By 10 min after the addition of T_3, this increase was no longer evident. Subsequent experiments, in which cellular cAMP was measured 5 min after the addition of T_3, revealed a dose-related stimulatory effect, evident at T_3 concentrations of 1 nM or more (Fig. 3).

Basal cAMP concentration in thymocytes was unaffected by incubation in calcium-free medium, but in this medium the increase in cellular cAMP concentration induced by 10 μM T_3, like the increase in 2-DG uptake, was completely abolished (Fig. 2).

In both standard and calcium-free medium, T_3 had no effect on medium cAMP concentration measured at the end of the incubation periods.

Effects of dibutyryl cAMP

In view of the ability of T_3 to increase thymocyte cAMP concentration, experiments were conducted to determine whether cAMP, specifically its analogue dibutyryl cAMP, would mimic the effect of T_3 on 2-DG uptake. Preincubation of thymocytes with dibutyryl cAMP (Bt_2cAMP) resulted in an increased thymocyte uptake of 2-DG. Both the magnitude and the time of onset of this response were dose related. Significant stimulation of 2-DG uptake (16±1.5%) was evident after only 5 min of preincubation with 1 mM Bt_2cAMP. but not with 0.1 mM Bt_2cAMP. However, 0.1 mM Bt_2cAMP was stimulatory when preincubated with thymocytes for 60 min. In studies of cells preincubated with various concentrations of Bt_2cAMP for 60 min,

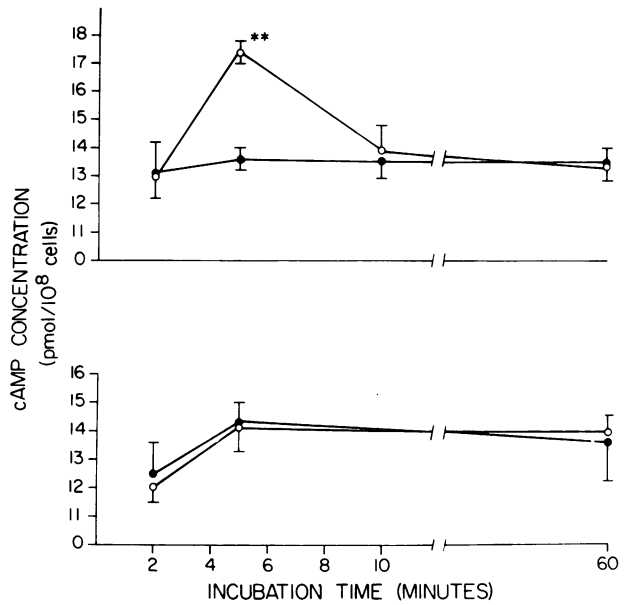

FIGURE 2 Calcium dependence of the stimulation of cAMP concentration in rat thymocytes by T_3. Cells were incubated in standard medium containing 1 mM Ca^{2+} (top) or in calcium-free medium (5 μM Ca^{2+}) (bottom). T_3, 10 μM (○), was then added and, at the periods indicated, its effect on cellular cAMP concentration was assessed. Values shown are mean±SD of those obtained in 3 to 12 separate experiments (3 at 2 min of incubation, 12 at 5 min, and 4 at 10 and 60 min), in which triplicate samples in each experimental group were studied. $^{**}P < 0.01$ vs. corresponding control group (●).

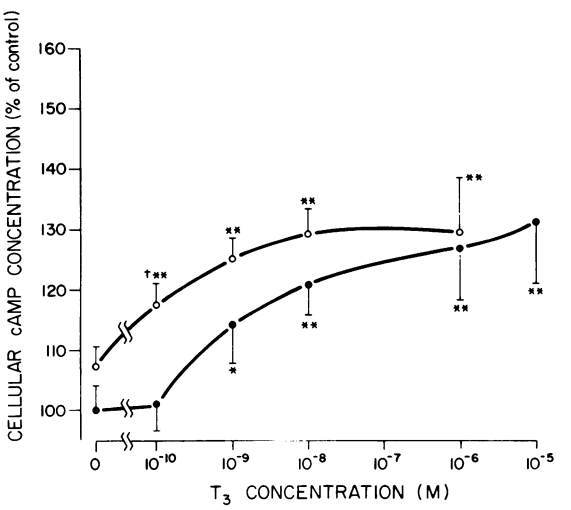

FIGURE 3 The effect of T_3 and epinephrine on cAMP concentration in rat thymocytes. Cells were incubated with various concentrations of T_3 in the presence (○) or absence (●) of 0.1 μM epinephrine. At the end of 5-min incubation period, the reaction was stopped and cellular cAMP concentration was assessed as described in Methods. Values shown are those obtained in five separate experiments in which triplicate samples in each experimental group were studied. $^{*}P < 0.05$ vs. corresponding control group, $^{**}P < 0.01$ vs. corresponding control group; $\dagger P < 0.05$ vs. the combined effect of the corresponding concentrations of T_3 and of epinephrine alone.

significant stimulation of 2-DG uptake again was produced by 0.1 mM Bt_2cAMP and maximum stimulation by 1 mM Bt_2cAMP (Fig. 4), with decreasing effects at concentrations >2 mM (data not shown). cAMP itself, in concentrations ranging from 1 μM to 10 mM, had no effect on 2-DG uptake, while butyric acid (1 mM) produced a slight inhibition (data not shown).

Unlike the effect of T_3, the stimulation of 2-DG uptake produced by Bt_2cAMP was not altered by the use of calcium-free medium (Fig. 4).

Effects of adrenergic agents

In previous experiments, we have shown that T_3 and epinephrine independently stimulate 2-DG uptake in rat thymocytes, and interact synergistically in this respect (1, 2). Therefore, experiments were performed to compare the properties of the stimulatory response of the 2-DG uptake to epinephrine with those of the response to T_3.

Effect on 2-DG uptake: lack of calcium dependence. In medium containing 1 mM Ca^{2+}, in confirmation of earlier findings (2), 1 and 10 μM epinephrine increased thymocyte uptake of 2-DG by 9.7±2.1 and 33.9±3.5%, respectively. In contrast to the findings with T_3, but like those with Bt_2cAMP, comparable increases were produced by both 1 and 10 μM epinephrine in calcium-free medium (14.6±1.9 and 33.1±5.3%). Similarly, calcium was not required for the stimulation of 2-DG uptake produced by the specific β-agonist, isoproterenol, in concentrations of 1 and 10 μM (data not shown).

Effect on thymocyte cAMP concentration. At a concentration of 10 μM, epinephrine significantly increased thymocyte cAMP concentration, an effect that was evident at 2 min, reached a maximum (34.0±2.8%, $P < 0.01$) at 5 min, and declined thereafter (Fig. 5). When measured at 5 min, both epinephrine and isoproterenol produced a dose-related stimulatory effect on cellular cAMP concentration, significant stimulation occurring at concentrations of 1 μM. These effects were calcium-independent (Fig. 6). Epinephrine did not affect the cAMP concentration in the suspend-

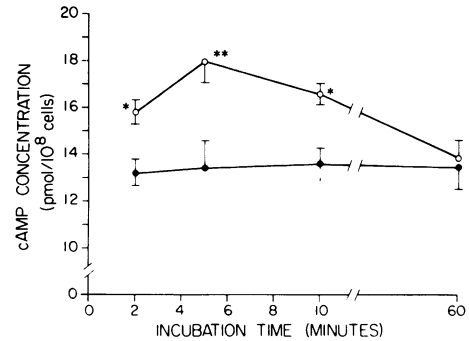

FIGURE 5 Enhancement of cAMP concentration in rat thymocytes by epinephrine. Cells were incubated in standard medium. Epinephrine (○) (10 μM) was then added, and, at the periods indicated, its effect on cellular cAMP concentration was evaluated as described in Methods. Values shown are mean±SD of those obtained in two or three separate experiments in which triplicate samples in each experimental group were studied. *$P < 0.05$ vs. corresponding control group (○); **$P < 0.01$ vs. corresponding control group.

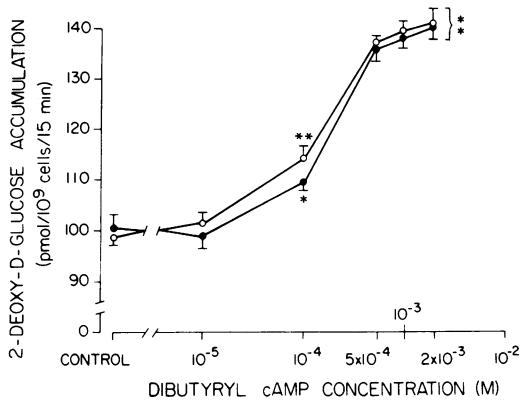

FIGURE 4 Stimulation of 2-DG uptake by Bt_2cAMP in rat thymocytes: lack of calcium dependence. Cells were incubated in a standard medium (●) or in calcium-free (○) medium. Bt_2cAMP was then added in the various concentrations indicated, and 60 min later in its effect on [^{3}H]2-DG was assessed as described in Methods. Values shown are mean±SD of those obtained in three separate experiments. * $P < 0.05$ vs. corresponding control; ** $P < 0.01$ vs. corresponding control.

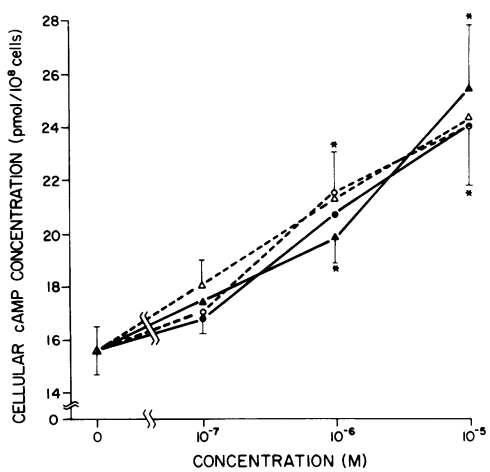

FIGURE 6 The effects of epinephrine and of isoproterenol on cAMP concentration in rat thymocytes: lack of calcium dependence. Cells were suspended in standard medium (-----) or in calcium-free medium (——). Various concentrations of epinephrine (●, ○) and of isoproterenol (▲, △) were then added, and 5 min later cellular cAMP concentration was determined as described in Methods. Values shown are those obtained in four separate experiments. * $P < 0.01$ vs. corresponding control group.

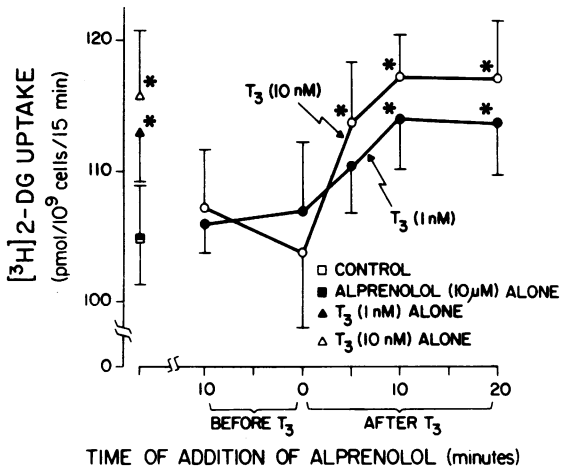

FIGURE 7 Time dependence of the inhibitory effect of alprenolol on the T_3-induced stimulation of 2-DG uptake in rat thymocytes. Cells were preincubated with and without T_3 (1 and 10 nM) in the presence and absence of alprenolol (10 μM), and 60 min later their 15-min [^{3}H]2-DG uptake was measured as described in Methods. In experiments in which the effect of T_3 in the presence of alprenolol was studied, T_3 was added at 0 time. Alprenolol was added prior to, together with, or after T_3; 60 min after the addition of T_3 its effect on [^{3}H]2-DG uptake was assessed. Values shown are mean±SD of those obtained in four separate experiments. * $P < 0.05$ vs. corresponding control group and groups in which alprenolol was given 10 min prior to or concomitant with T_3.

ing medium, measured at the end of incubation periods.

Low concentrations of epinephrine (0.1 μM) failed to increase significantly the thymocyte concentration of cAMP. However, when this concentration of epinephrine was combined with an ineffective concentration of T_3 (0.1 nM), a highly significant increase in cellular cAMP concentration was observed (Fig. 3). At increasing concentrations of T_3, this effect of epinephrine progressively diminished. Phenylephrine, a specific α-agonist, in concentrations as high as 1 mM, had no effect on thymocyte cAMP concentration (data not shown).

Effects of alprenolol

Effect on 2-DG responses of T_3 and Bt_2cAMP. In confirmation of previous results (2), 10 μM L-alprenolol[3] added to thymocyte suspensions either 10 min prior to or concomitant with T_3 (1 or 10 nM) abolished the stimulatory effect of T_3 on 2-DG uptake. In contrast, when added 5 min after T_3, alprenolol only partly inhibited the response to T_3; when added 10 min or more after T_3, it was entirely ineffective (Fig. 7). D-Alprenolol (0.1 mM), on the other hand, had no effect on basal 2-DG uptake and had no effect on the 2-DG response to T_3 (data not shown).

Preincubation of thymocytes with 10 μM alprenolol for 15 min did not alter significantly the increase in 2-DG uptake seen either 5 or 60 min after the addition of 0.1 or 1 mM Bt_2cAMP (Table I).

Effects on cAMP responses to T_3 and adrenergic agents. As with the stimulation of 2-DG uptake produced by T_3 or epinephrine, the increase in thymocyte cAMP concentration induced by these agents was inhibited by alprenolol. Alprenolol alone (10 μM) had no effect on thymocyte cAMP concentration. However, when added either 15 min before or concomitant with 1 or 10 nM T_3, the increase in cAMP concentration seen 5 min after the addition of T_3 was abolished or decreased, respectively (Fig. 8). Entirely analogous results were evident when alprenolol was allowed to interact with 1.0 and 10 μM epinephrine.

Alprenolol (10 μM) also proved capable of inhibiting the increase in thymocyte cAMP concentration induced by 1 and 10 μM isoproterenol (data not shown).

DISCUSSION

We have previously suggested that the T_3-induced increase in the uptake of 2-DG and 3-*O*-MG by rat

TABLE I

*The Effect of Bt_2cAMP, in the Presence and Absence of Alprenolol, on 2-DG Uptake in Rat Thymocytes**

Preincubation time with Bt_2cAMP	Bt_2cAMP	Alprenolol	[^{3}H]2-DG uptake	Effect
min	*mM*	*μM*	*pmol/10⁹ cells/15 min*	*% control*
5	—	—	97.1±3.2	—
5	—	10	95.3±3.3	98.1
5	0.1	—	100.7±5.2	103.7
5	0.1	10	97.4±3.9	100.3
5	1.0	—	115.7±5.9‡	119.2
5	1.0	10	115.2±5.4‡§	118.6
60	—	—	96.3±3.6	—
60	—	10	97.2±5.2	100.9
60	0.1	—	110.4±4.5‡	114.6
60	0.1	10	111.1±3.4‡§	115.4
60	1.0	—	131.7±4.4‡	136.8
60	1.0	10	129.4±6.9‡§	134.4

* Thymocytes were suspended in standard medium. Bt_2cAMP and alprenolol, at the indicated concentrations, were then added, and 5 or 60 min later the 15-min uptake of [^{3}H]2-DG by the cells was measured as described in Methods. Values shown are mean±SD of those obtained in four separate experiments.

‡ $P < 0.02$ vs. corresponding groups incubated without Bt_2cAMP.

§ Values not significantly different from corresponding groups not treated with alprenolol.

[3] In this discussion, unless otherwise indicated, the term "alprenolol" refers to the levo-isomer.

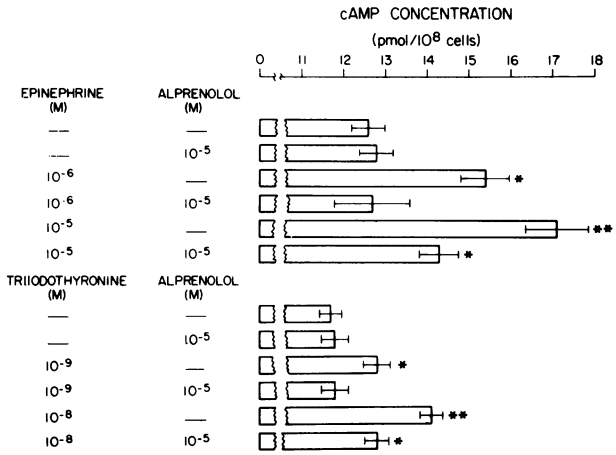

FIGURE 8 The effects of epinephrine and of T_3, in the presence or absence of alprenolol, on the concentration of cAMP in rat thymocytes. Cells were incubated with various concentrations and combinations of epinephrine and alprenolol (upper panel) and T_3 and alprenolol (lower panel). At the end of the 5-min incubation period, cellular cAMP concentration was determined as described in Methods. Values shown are mean±SD of those obtained in three separate experiments in which triplicate samples in each experimental group were studied. The experiments with T_3 and those with epinephrine were performed on separate days. This may explain the difference in the basal 2-DG uptake values between the two groups. *$P < 0.05$ vs. corresponding control group; **$P < 0.01$ vs. corresponding control group.

thymocytes in vitro reflects a primary action of thyroid hormone at the level of the plasma membrane that enhances the inward transport of sugar (1, 2). The present studies, we believe, provide strong evidence for this view and shed light on the antecedent events that seem to be part of the mechanism of the T_3 effect. They indicate that T_3 increases the transport of the sugar into the cell by producing a calcium-dependent increase in thymocyte cAMP concentration. The mechanism by which this increase in thymocyte cAMP concentration is brought about is uncertain, but is the subject of ongoing experiments.[4]

The evidence upon which this hypothesis is based is consistent with the criteria generally accepted as evidence for a cAMP-mediated effect. First, T_3 increased thymocyte cAMP concentration in a dose-dependent manner. The effect of T_3 on cAMP concentration preceded the stimulation of 2-DG uptake, and the minimum concentration required to increase the cAMP concentration was the same as that required to enhance the transport of 2-DG. Second, the effect of T_3 was mimicked by the addition of Bt_2cAMP, and the interval between the addition of this agent and the stimulation of 2-DG uptake that it produced was less than that seen in the case of T_3. Third, when calcium was omitted from incubation medium, T_3 failed to increase thymocyte cAMP concentration and failed to stimulate 2-DG transport. Similarly, both responses to T_3 were blocked by the addition of alprenolol,[5] and the concentrations of alprenolol required to inhibit the two effects were the same. It is extremely unlikely that these coordinate effects of alprenolol represent some nonspecific effect on the plasma membrane, since the concentrations of alprenolol required to inhibit the response to T_3 were themselves without any effect on 2-DG uptake, and since D-alprenolol did not display the inhibitory effect shown by the L-isomer. Further, alprenolol did not prevent the effect of T_3 on 2-DG uptake when added after the transient effect of T_3 on cAMP concentration was complete, and neither did it alter the stimulatory response of the 2-DG uptake to Bt_2cAMP.

Consistent with the hypothesis that T_3 acts on thymocyte 2-DG uptake by increasing the concentration of cAMP is the fact that epinephrine, which also increases thymocyte cAMP concentration, also stimulates the uptake of 2-DG. Both effects of epinephrine are blocked by alprenolol. It seems reasonable to suggest, therefore, that the independent stimulatory effects of T_3 and of epinephrine on 2-DG uptake are both mediated by the increase in cellular cAMP that they induce. Furthermore, when T_3 and epinephrine are added together, the increase in cellular cAMP concentration is greater than that seen when either agent is added alone. This may account for the synergistic interaction between the two agents with respect to stimulation of 2-DG uptake. The properties of the cAMP response to the two agents differ, however, since the response to T_3 is calcium-dependent, whereas that to epinephrine is not.

There exists a very extensive literature concerning the effects of various hormones and other agents on cellular calcium, cyclic nucleotide, and sugar metabolism, and their relationship to one another. A review of all these studies is far beyond the scope of this report. There are, however, a number of findings that seem to have particular relevance to the actions of T_3 that we have described, and that consequently merit considera-

[4] Studies to date have revealed that the proportionate increase in thymocyte cAMP concentration produced by T_3 is the same in the presence and absence of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine. This suggests that T_3 does not increase cellular cAMP concentration by blocking phosphodiesterase activity.

[5] The inhibitory effect of alprenolol on these responses to T_3 is reminiscent of that reported by Popovic and co-workers (40), who demonstrated that T_4 in vitro increases the erythroid colony-forming activity of canine marrow cells, and that this stimulation is inhibited by propranol. Apart from these two responses, we know of no other system in which an effect of thyroid hormones that occurs in the absence of catecholamines is blocked by an adrenergic antagonist.

tion. These are the effects of T_3 on cellular cAMP concentration and the general interrelation between calcium and cAMP metabolism.

Apparent contradictions exist with respect to the in vitro effect of thyroid hormones on the cAMP concentration of intact cells. Many studies in cells from different tissues have indicated that thyroid hormones are inactive in this regard (28–33). On the other hand, T_3 in vitro has been found to increase the cAMP concentration in spermatozoa from *Macaca mulatta* (34), and thyroxine to act similarly in rat osteogenic sarcoma cells in culture (17). The latter findings are obviously in accord with our own findings in the rat thymocyte.

In addition, our data, which suggest that there is a relationship between calcium and the effect of T_3 on thymocyte cAMP metabolism, are supported by analogous findings in other systems. Thus, the ACTH action to increase tissue cAMP concentration in both adrenal cortex and adipose tissue is calcium dependent (35–37). Further, in lymphoid cells in vitro, the mitogen concanavalin A induces calcium-dependent increases in the uptake of both 3-*O*-MG and glucose (38, 39).

Thus, in a general way, the literature offers some support to the conclusions drawn from this study, that T_3 enhances sugar uptake in the rat thymocyte via a calcium-dependent increase in cellular cAMP concentration. The promptness of this response, its independence of new protein synthesis, its direct inhibition by alprenolol, and its similarity to the response to epinephrine all suggest that this effect of T_3 is mediated at the plasma membrane. This conclusion is supported by our recent demonstration of specific binding sites for T_3 in highly purified thymocyte plasma membranes, a finding that will be the subject of a later report.

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