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Research Article

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ABSTRACT We have previously demonstrated that 3,5,3′-triiodothyronine (T_3), whether administered in vivo or added to suspending media in vitro, promptly stimulates the in vitro accumulation of the non-metabolized amino acids, alpha-aminoisobutyric acid, and cycloleucine (CLE) by thymocytes isolated from weanling rats. In these studies, we have examined the in vitro interaction between catecholamines and T_3 with respect to this effect. The previously reported enhancement of CLE accumulation in thymocytes by T_3 in vitro (1 μM) was confirmed. When added alone in concentrations ranging between 10 nM and 0.1 mM, the adrenergic agonists, epinephrine and norepinephrine, had no effect on CLE accumulation. At a concentration of 1 μM, isoproterenol, terbutaline, and phenylephrine were also without effect. However, the effect of T_3 was clearly potentiated by the concomitant addition of epinephrine, norepinephrine, and possibly isoproterenol, whereas terbutaline and phenylephrine were without effect. Neither basal nor T_3-enhanced CLE accumulation was affected by the addition alone of the adrenergic blocking agents, propranolol (0.1 mM), phentolamine (10 μM), or practolol (0.1 mM). Nevertheless, the $beta_1$- and $beta_2$-antagonist, propranol, and the $beta_1$-antagonist, practolol, blocked the increment in CLE accumulation produced by epinephrine; the alpha-antagonist, phentolamine, was without effect.

The enhancement of CLE accumulation that occurred in the presence of T_3, with or without epinephrine, was seen to be a result of an inhibition of CLE efflux, because T_3 alone inhibited CLE efflux, and this effect was increased when epinephrine was also present. On the other hand, neither T_3 alone nor T_3 plus epinephrine appreciably altered the rate of inward transport of CLE. As judged from studies of the ability of thymocytes to exclude trypan blue, neither T_3 alone nor T_3 plus epinephrine either enhanced or impaired viability of cells during 3-h periods of incubation. Cell water content, measured with [^{3}H]urea, was unaffected by T_3, either alone or in the presence of epinephrine. In confirmation of previous results, the stimulatory effect of T_3 on CLE accumulation was unaffected by concentrations of puromycin sufficient to inhibit protein synthesis by at least 95%, and the potentiating action of epinephrine on the response to T_3 was similarly unaffected.

From these findings, it is concluded that the effect of T_3 to increase CLE accumulation by thymocytes in vitro, though itself independent of adrenergic mediation, is potentiated by $beta_1$-adrenergic stimulation. This interaction appears distinctly different from other thyroid hormone-catecholamine interactions, in which thyroid hormones enhance physiological responses to catecholamines. Its mechanism remains unclear, but the properties of the T_3 effect, and possibly the interaction itself, suggest that T_3 enhances CLE accumulation by an action at the level of the cell membrane.

INTRODUCTION

A number of questions concerning the actions of the thyroid hormones have long been under investigation but remain, nevertheless, incompletely clarified. Among these are questions concerning the primary mechanism of thyroid action at the cellular and subcellular levels; others concern the nature of the interaction between thyroid hormones and catecholamines that is so apparent in many physiological and clinical settings (1–3). With respect to the primary locus of thyroid hormone action, numerous data suggest

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a direct action at the nuclear level to modify gene expression (4–7). Still other data raise the possibility that high affinity receptors for thyroid hormone exist within the mitochondrion and that thyroid hormones exert a direct effect on mitochondrial function (8–10). Data recently acquired by ourselves and others also support a direct, extranuclear action of thyroid hormones. Thus, in vitro, thyroid hormones enhance the accumulation of the nonmetabolized amino acids, alpha-aminoisobutyric acid, and 1-amino-cyclopentane-1-carboxylic acid (cycloleucine [CLE])[1] by both chick embryo cartilage (11, 12) and rat thymocytes (13, 14), and also increase the transport of the glucose analogue, 2-deoxy-D-glucose, by chick embryo myocardium in culture (15) and by freshly isolated rat thymocytes (16). These effects of thyroid hormone appear very promptly and are unaffected by inhibitors of protein or RNA synthesis, or both. Both the nature and promptness of these effects point to an action of thyroid hormone on the cell membrane or on membrane-mediated function.

In these studies, we have extended our earlier observations concerning the stimulatory effect of 3,5,3′-triiodothyronine (T_3) on the accumulation of CLE by rat thymocytes in vitro and have explored the interactions between T_3 and catecholamines within this system. The data reveal a previously unknown and possibly unique interaction in which certain catecholamines, though themselves without effect, act promptly to enhance the increase in CLE accumulation produced by T_3.

METHODS

Experiments were performed in thymocytes isolated from female CD rats purchased from Charles River Breeding Laboratories, Wilmington, Mass. Animals were 21 d old at the time of arrival and were fed Purina Laboratory Chow (Ralston Purina Co., Inc., St. Louis, Mo.) and given tap water to drink ad libitum until the time of sacrifice, which occurred between 2 and 8 d after arrival of animals in the laboratory.

On the day of each experiment, a single rat was decapitated and allowed to exsanguinate. The thymus gland was then quickly but gently removed, and thymocytes were isolated by the general technique previously employed in this laboratory (17). The gland was placed into a Petri dish standing on crushed ice and containing ice-cold buffer. The buffer employed contained the following: 120 mM NaCl; 5 mM KCl; 1 mM $CaCl_2$; 2.5 mM $MgCl_2$; 1.5 mM NaH_2PO_4; 25 mM Tris-HCl; 20 mM Hepes; and 22 mM glucose at pH 7.5.

Cells were freed from the thymus by gentle teasing with forceps, and when the liquid phase had become cloudy it was aspirated and filtered through nylon mesh. The filtrate was then centrifuged at 100 *g* for 20 min at 4°C, the supernate discarded, and the thymocyte pellet resuspended in 20 ml of cold buffer. Cells were then counted in a hemocytometer, and buffer was added to yield a final thymocyte concentration of $2–4 \times 10^7$ cells/ml.

Accumulation of CLE. 1-ml aliquots of cell suspension were pipetted into Erlenmeyer flasks and were allowed a 15-min period of equilibration in a metabolic shaker at 37°C under room air. At this time, 50-μl aliquots of buffer which contained T_3, adrenergic agonists, or adrenergic antagonists[2] were added in varying combinations to the experimental flasks while equal volumes of buffer were added to the controls. Incubations were then continued for an additional 30 min. At that time, 50 μl of a solution of [^{14}C]CLE was added to all vessels, together with 50 μl of a [^{3}H]mannitol solution to serve as an extracellular marker. Final concentration of CLE was 30 μM, and that of mannitol was 4 nM. In all experiments, duplicate vessels for each experimental group and for the control group were prepared.

Suspensions were then incubated for 2 h, at which time triplicate 100-μl aliquots were pipetted from each flask and transferred to microfuge tubes. Suspensions were then centrifuged for 15 s at 10,000 *g* in a Beckman microfuge (Beckman Instruments Inc., Spinco Div., Palo Alto, Calif.), the supernates were carefully aspirated, and 100 μl of 10% perchloric acid was added to the cells. Each tube was then agitated vigorously in a Vortex mixer (Scientific Industries, Inc., Bohemia, N. Y.), and its contents were transferred to a scintillation vial. Each tube was washed with scintillation fluid, which was then transferred into its corresponding vial, and samples were counted in a liquid scintillation counter.

Uptake of CLE by thymocytes was calculated from the ^{14}C counts contained in each vial, the known total counts added, and the known final concentration of CLE in the medium. Values for the uptake were corrected for the volume of the occluded extracellular phase, as judged from the ^{3}H counting rate, and corrected values for CLE uptake were expressed as nanomoles per 10^9 cells per 2 h.

Influx and efflux of CLE. Experiments were performed to ascertain the effect of various agents on the rate of influx of CLE into thymocytes. In these experiments, incubations were carried out exactly as described above, except that they were terminated 5 min after the addition of [^{14}C]CLE.

Other experiments were performed to assess the effect of various agents on the rate of efflux of accumulated [^{14}C]CLE from thymocytes. In these experiments, large volumes of thymocyte suspensions in media containing the agents to be tested were incubated with [^{14}C]CLE for 1 h. Three 1-ml aliquots from each of the experimental groups were then pipetted into centrifuge tubes and spun for 5 min at 2,000 *g*. The pellets were then resuspended in buffer that contained the same agents with which the thymocytes had originally been incubated, but lacking [^{14}C]CLE. Immediately, and at 5-min intervals thereafter, 100-μl aliquots were removed, centrifuged, and prepared for counting as in the accumulation experiments described above.

Effect of puromycin. Four experiments were performed in which the effect of puromycin on the response of CLE accumulation by thymocytes to T_3, with or without

[1] *Abbreviations used in this paper*: CLE, cycloleucine; T_3, 3,5,3′-triiodothyronine.

[2] The following reagents were obtained from commercial sources: [^{14}C]CLE and [^{3}H]mannitol were from New England Nuclear, Boston, Mass.; T_3, propranolol, epinephrine, norepinephrine, phenylephrine, and isoproterenol were from Sigma Chemical Co. St. Louis, Mo. Phentolamine was a gift from the Ciba-Geigy Corp., Pharmaceuticals Div., Summit, N. J., practolol was from Ayerst Laboratories, N. Y., and terbutaline was from Astra Pharmaceutical Products, Inc., Framingham, Mass. Optically active compounds among those listed above were all studied in the levoratory form.

epinephrine, was assessed. Cells were preincubated for 15 min, after which puromycin was added to one-half of the vessels at a concentration (100 μg/ml) previously shown to inhibit amino acid incorporation into thymocyte protein under similar conditions of incubation by 95% (16). After 10 min, certain of the vessels were enriched with T_3 (1 μM) or T_3 plus epinephrine (1 μM). 30 min later, [^{14}C]CLE and [^{3}H]mannitol were added to all vessels, as described above. Aliquots of cell suspensions were removed for measurement of CLE accumulation 30 min and 2 h after addition of CLE. In each of these experiments, all experimental groups were studied in quadruplicate.

Cell integrity and viability. Additional experiments were performed to assess the effects of T_3, with or without epinephrine, on cellular integrity and viability. Four experiments were performed in which triplicate vessels containing thymocytes were incubated for varying periods with or without T_3 (1 μM) or T_3 plus epinephrine (1 μM), together with [^{3}H]mannitol, as above, and [^{14}C]urea (4 μCi/ml; 47.1 mCi/nmol sp act). At the end of incubations, cells were processed for the measurement of intracellular [^{14}C]urea content as for the measurement of [^{14}C]CLE content, described above.

In other experiments, control vessels and vessels containing either T_3 (1 μM) or T_3 plus epinephrine (1 μM) were incubated for 3 h with trypan blue (0.3%). Aggregation or disaggregation of the cells, as well as their ability to exclude trypan blue, was then assessed microscopically.

RESULTS

Effect of adrenergic agonists (Table I). Epinephrine and norepinephrine, in concentrations ranging between 10 nM and 100 μM, as well as isoproterenol, terbutaline, and phenylephrine at 1 μM, all failed to induce a significant change in CLE accumulation.

Effect of adrenergic agents on the action of T_3 (Table II). Studies were performed to ascertain whether the adrenergic agonists, though inactive alone, would alter the increase in CLE accumulation in thymocytes induced by T_3. In these groups of experiments, with one exception, T_3 was employed at a concentration of 1.0 μM, and a similar concentration was employed in the case of the adrenergic agonists. The previously described effect of 1.0 μM T_3 to increase CLE accumulation was again observed, and both epinephrine and norepinephrine significantly increased CLE accumulation to values higher than those seen with T_3 alone. In the case of isoproterenol, values for CLE accumulation in the presence of both T_3 and isoproterenol were higher than those found with T_3 alone in each of the six experiments performed. However, the magnitude of this difference was quite variable, with the result that mean values were not significantly different by the paired *t* test that was employed in statistical analyses (18). Terbutaline and phenylephrine were entirely without effect.

Three experiments were performed in which a lower dose of T_3 (0.1 μM) was employed. As expected, no effect of T_3 alone on CLE accumulation was observed, but, despite the small number of experiments performed, CLE accumulation in the presence of epinephrine was significantly higher than in control vessels.

Effect of adrenergic antagonists (Table III). Experiments were also performed to determine the effect of adrenergic antagonists on both basal and T_3-stimulated CLE accumulation. Propranolol (0.1 mM), phentolamine (10 μM), and practolol (0.1 mM) all failed to influence basal CLE accumulation. Concen-

TABLE I

The In Vitro Effect of Adrenergic Agonists on CLE Accumulation by Rat Thymocytes

		CLE accumulation					
		Concentration of agonist					
Agonist	n*	0	10 nM	0.1 μM	1 μM	10 μM	0.1 mM
		nmol/10^6 cells/2 h					
Epinephrine	5	12.1±1.6‡	11.5±1.5	11.4±1.6	12.0±1.4	11.1±1.4	11.6±1.4
	8	10.3±1.5			10.3±1.4		
Norepinephrine	3	9.5±1.2	9.9±2	8.2±1.4	10.2±1.9	10.1±1.8	9.9±1.7
	4	9.4±2.1		8.6±1.3	9.8±1.7	9.8±1.6	9.9±1.2
	6	9.1±0.7			9.6±1.1		
Isoproterenol	3	8.3±1.6			7.7±0.8		
Terbutaline	5	10.1±0.3			10.2±0.4		
Phenylephrine	5	7.7±0.7			6.9±0.8		

* Number of experiments performed in which all the variables shown were tested in duplicate at the same time in identical thymocyte suspensions.

‡ Mean±SE. For each agonist, none of the values shown is significantly different from control or from any of the others.

TABLE II

*The Effect of Adrenergic Agonists on T_3-Stimulated Accumulation of CLE by Rat Thymocytes**

Agonists	n	CLE accumulation			Statistical analysis‡		
		(a) Control	(b) T_3	(c) T_3 + agonist	(b) − (a)	(c) − (a)	(c) − (b)
		nmol/10⁶ cells/2 h			*mean difference±SEMD*		
Epinephrine, 1 μm§	3	8.4±1.3	8.1±0.9	10.2±1.4	−0.4±0.6 NS	1.7±0.3 $P < 0.05$	2.0±0.4 NS
Epinephrine, 1 μM	20	9.6±0.6	11.8±0.9	13.8±1.2	2.1±0.7 $P < 0.01$	4.2±0.8 $P < 0.01$	2.1±0.6 $P < 0.01$
Norepinephrine, 1 μM	5	8.5±1.1	11.3±1.4	14.1±1.3	2.8±0.9 $P < 0.05$	5.6±1.2 $P < 0.05$	2.8±0.9 $P < 0.05$
Isoproterenol, 1 μM	6	9.8±0.6	13.1±1.1	14.5±2.2	3.3±0.7 $P < 0.01$	4.7±1.8 $P < 0.05$	1.4±1.3 NS
Terbutaline, 1 μM	6	10.1±0.7	13.7±0.8	13.5±0.7	3.6±0.3 $P < 0.02$	3.4±0.1 $P < 0.01$	−0.1±0.4 NS
Phenylephrine, 1 μM	5	7.5±1.4	9.9±1.2	9.4±1.2	2.4±0.4 $P < 0.01$	1.8±0.3 $P < 0.01$	−0.4±0.1 NS

* Values for CLE accumulation shown are mean±SE for the number of separate experiments, indicated by *n*, in which all variables shown were tested in duplicate at the same time in identical thymocyte suspensions.

‡ Statistical analyses by paired *t* test.

§ In these experiments, T_3 was added at a concentration of 0.1 μM. In the remaining experiments, the concentration of T_3 employed was 1 μM.

trations of these agents lower than the above were also without effect (data not shown). Results shown for phentolamine are those obtained with a concentration of 10 μM, because a 0.1-mM concentration of this agent inhibited basal CLE accumulation.

In experiments seeking a direct interaction between T_3 and the adrenergic blocking agents, significant stimulation of CLE accumulation by T_3 alone was observed in those experiments with propranolol and phentolamine. In each of four experiments concerning the effects of practolol, T_3 increased CLE accumulation above basal values, but owing to variation in the magnitude of the response to T_3, the effect of T_3 in these experiments was not statistically significant.

As had been the case with basal CLE accumulation (Table II), none of the adrenergic antagonists appreciably affected the increased CLE accumulation observed in the presence of T_3.

Effect of adrenergic antagonists in the presence of T_3 plus epinephrine (Table IV). Experiments were then conducted to ascertain whether the adrenergic antagonists would influence the synergistic interaction between T_3 and epinephrine already demonstrated. In these experiments, CLE accumulation was measured in control vessels, as well as in vessels containing T_3 (1 μM), T_3 plus epinephrine (1 μM), and T_3 plus both epinephrine and adrenergic antagonist. The concentrations of antagonists employed were the highest concentrations previously shown to be without effect on either basal or T_3-increased CLE accumulation.

In these experiments, the well-documented effect of T_3 to increase CLE accumulation was again observed (statistical significances not shown), and in each of the three groups of experiments dealing with the three antagonists studied, CLE accumulation in the presence of T_3 plus epinephrine was significantly greater than that seen with T_3 alone. Phentolamine (10 μM) had no effect on the accumulation of CLE seen in the presence of T_3 plus epinephrine, with values remaining significantly higher than those seen in the presence of T_3 alone. In contrast, propranolol (0.1 mM) and practolol (0.1 mM) both blocked the potentiating effect of epinephrine on the action of T_3, significantly reducing CLE accumulation to values almost identical to those seen with T_3 alone.

Effect of T_3 with and without epinephrine on CLE influx and efflux. In four experiments in which thymocytes were allowed to accumulate [^{14}C]CLE in control vessels, others containing T_3 (1 μM), and still others containing T_3 plus epinephrine (1 μM), the rate of efflux of [^{14}C]CLE was assessed after transfer of thymocytes to analogous media devoid of [^{14}C]CLE. In each of the four experiments, the rate of efflux of [^{14}C]CLE was greatest in control samples, less in samples containing T_3, and least in samples containing T_3 plus epinephrine. Covariance analysis (18) of pooled

TABLE III

*The Effect of Adrenergic Antagonists on T_3-Stimulated CLE Accumulation by Rat Thymocytes In Vitro**

Antagonists	n	CLE accumulation (a) Control	(b) T_3	(c) Antagonist	(d) Antagonist + T_3	Statistical analysis‡ (b) − (a)	(c) − (a)	(d) − (b)
		nmol/10⁹ cells/2 h				*Mean difference±SEMD*		
Propranolol, 0.1 mM	8	7.6±0.3		7.2±0.2			−0.5±0.2 NS	
	8	8.2±0.5	10.0±0.6		9.5±0.7	1.8±0.5 $P < 0.01$		−0.4±0.5 NS
Phentolamine, 10 μM	3	11.4±3.1		10.6±2.9			−0.7±0.4 NS	
	6	10.6±1.4	12.3±1.3		12.1±1.3	1.7±0.4 $P < 0.02$		−0.2±0.2 NS
Practolol, 0.1 mM	6	9.6±0.6		9.3±0.9			−0.3±0.6 NS	
	4 4	9.6±0.7	12.8±1.7		12.2±1.9	3.2±1.2 NS		−0.6±0.6 NS

* Values for CLE accumulation are mean±SE for the number of experiments, indicated by *n*, in which all variables shown were tested in duplicate at the same time in identical thymocyte suspensions. T_3 was employed at a concentration of 1 μM.
‡ Statistical analyses by paired *t* test.

data from the four experiments revealed significant differences in the efflux rates among all three experimental groups (Fig. 1).

No significant effect of T_3, with or without epinephrine, on the influx of [^{14}C]CLE into thymocytes was observed. In six experiments, the accumulation of CLE after 5 min averaged 6.0±2.4 (nanomoles per 10^9 cells; mean±SD) in control specimens, 6.4±2.9 in specimens incubated with T_3 (1 μM), and 5.2±2.4 in specimens incubated with T_3 plus epinephrine (1 μM).

Effect of puromycin. In four experiments, preincubation with puromycin (100 μg/ml) inhibited the 30-min accumulation of CLE in T_3- and epinephrine-free control thymocytes by 11.4±0.3%, and the

TABLE IV

*Effect of Adrenergic Antagonists on the Stimulation of CLE Accumulation in Rat Thymocytes Produced by T_3 and Epinephrine**

Antagonist	n	CLE accumulation (a) Control	(b) T_3	(c) T_3 + Epi§	(d) T_3 + Epi + Antag	Statistical analysis‡ (c) − (b)	(d) − (b)	(d) − (c)
		nmol/10⁹/2 h				*Mean difference±SEMD*		
Propranolol, 0.1 mM	6	10.6±0.7	13.0±1.4	18.2±2.5	12.6±1.6	5.2±1.6 $P < 0.05$	−0.4±0.6 NS	−5.6±1.6 $P < 0.02$
Phentolamine, 10 μM	4	10.7±2.2	12.4±2.0	13.9±1.9	13.9±2.1	1.5±0.2 $P < 0.01$	1.5±0.2 $P < 0.01$	0.0±0.2 NS
Practolol, 0.1 mM	5	10.0±0.7	12.7±1.4	15.2±1.4	12.2±1.5	2.5±0.8 $P < 0.05$	−0.6±0.4 NS	−3.1±1.1 $P < 0.05$

* Values for CLE accumulation are mean±SE for the number of separate experiments, indicated by *n*, in which all the variables shown were tested in duplicate at the same time in identical thymocyte suspensions. T_3 and epinephrine were employed at concentrations of 1 μM, antagonists were tested at the concentration indicated.
‡ Statistical analyses by paired *t* test.
§ Epi, epinephrine; Antag, antagonist.

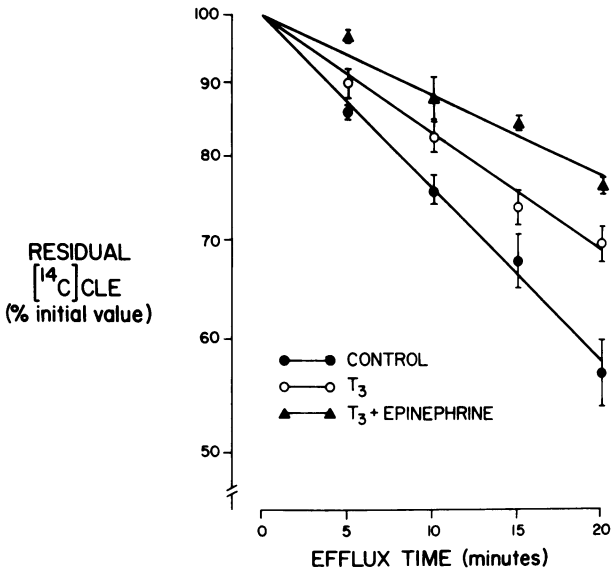

FIGURE 1 The effect of T_3, with and without epinephrine, on the efflux of [^{14}C]CLE from rat thymocytes in vitro. Thymocytes were incubated with [^{14}C]CLE at 37°C for 1 h in control medium, medium containing T_3 (1 μM), or medium containing T_3 plus epinephrine (1 μM). Cells were then transferred to analogous media that lacked [^{14}C]CLE. Aliquots of suspensions were removed immediately and at 5-min intervals thereafter for measurement of cellular [^{14}C]CLE content. Results shown are the means of those obtained in four experiments in which duplicate samples from each group were studied. Covariance analysis (18) revealed significant differences ($P < 0.01$) among efflux slopes of all three groups.

2-h accumulation of CLE by 48.9±1.6%. Nevertheless, puromycin did not alter the proportionate stimulation of CLE accumulation produced by T_3 (1 μM) or T_3 plus epinephrine (1 μM) at either time period. In the absence of puromycin, T_3 increased the 30-min accumulation of CLE significantly, by 27.7±4.5%; the percentage of increase (25.3±3.2%) was almost identical in the cells treated with puromycin. A significantly ($P < 0.01$) greater proportionate enhancement of 30-min CLE accumulation was seen in vessels containing T_3 plus epinephrine (38.0±6.8%), and this increase (38.1±6.5%) was also uninfluenced by puromycin.

Similar results were obtained with respect to the 2-h accumulation of CLE. T_3 increased CLE accumulation by 31.0±2.8% in the absence of puromycin and by 29.2±5.4% in the presence of puromycin. A significantly ($P < 0.05$) greater increment in CLE accumulation was produced by T_3 in the presence of epinephrine (40.8±3.8%), and this too was unaltered by puromycin (39.3±5.8%).

Cell integrity and viability. Effects of T_3 (1 μM) and of T_3 plus epinephrine (1 μM) on CLE accumulation could not be explained by changes in the intracellular diffusion space of CLE, i.e., the intracellular water content of thymocyte suspensions, as judged from intracellular:extracellular ratios of [^{14}C]urea. In four experiments in which cells were incubated with or without T_3, epinephrine, or T_3 plus epinephrine for 2 h, ratios expressed as a percentage of those found in untreated controls were 99.3±3.1 in cells exposed to T_3 alone, 100.6±3.0 in cells exposed to epinephrine alone, and 95.7±1.0 in cells exposed to epinephrine plus T_3.

After 3 h of incubation, thymocytes remained unaggregated in control vessels, as well as in those containing T_3 or T_3 plus epinephrine, and trypan blue was excluded by 93–96% of cells in all three experimental groups.

DISCUSSION

In these studies we have confirmed our earlier observations indicating that T_3 in vitro is capable of enhancing the accumulation of the nonmetabolized amino acids, alpha-aminoisobutyric acid and CLE, by thymocytes harvested from weanling rats, and that this effect is mainly a result of an inhibition of amino acid efflux, rather than an enhancement of inward transport (13, 14). We have, in addition, demonstrated that a variety of adrenergic agonists, thought to be active at either the alpha- or beta-adrenergic receptor, do not in themselves, over a wide range of concentrations, significantly affect CLE accumulation. Despite this, certain of the adrenergic agonists, notably epinephrine and norepinephrine, proved capable of increasing the response to T_3, an effect seen to result from an increase in the T_3-mediated inhibition of CLE efflux. Moreover, at the concentrations tested, several adrenergic antagonists were themselves without effect on basal or T_3-increased CLE accumulation, but propranolol and practolol among them proved capable of abolishing the synergistic effect of epinephrine.

Among the variety of reported interactions between the thyroid hormones and adrenergic agonists (1–3), the presently described response appears, at least superficially, to be unique. Previously described interactions, certain of which are discussed more fully below, can be categorized as reflecting an enhanced response to catecholamines in the presence of excess or additional thyroid hormone and a depressed response in states of thyroid hormone lack. It is generally stated, therefore, that thyroid hormones increase the sensitivity of certain physiological systems that are responsive to catecholamines (2, 3). In these studies of the in vitro accumulation of CLE by thymocytes, in contrast, T_3 was stimulatory when added alone, and its effect was increased by the addition of epinephrine (or norepinephrine). However, concentrations of

epinephrine 100 times higher than those which increased the effect of T_3 failed to affect CLE accumulation when added alone. In this system, therefore, catecholamines potentiated the response to thyroid hormones, rather than vice versa.

As judged from conventional criteria (19), the potentiating effect of the catecholamines on this action of T_3 appeared to be mediated at the $beta_1$-receptor. A consistent enhancement of the T_3 effect was produced by both epinephrine and norepinephrine, which interact with both alpha- and beta-receptors. However, phenylephrine, which is solely an alpha-agonist, did not increase the effect of T_3; and phentolamine, an alpha-antagonist, did not inhibit the potentiating action of epinephrine. Moreover, the effect of epinephrine was abolished by propranol, a $beta_1$- and $beta_2$-antagonist. Finally, mediation at the beta-receptor was indicated both by the ability of practolol, a $beta_1$-blocking agent, to abolish the effect of epinephrine and by the failure of terbutaline, a $beta_2$-agonist, to potentiate the effect of T_3.

The promptness of the interaction between T_3 and catecholamines that we have described is not without precedent in previous reports. In rat epididymal adipose tissue, enhancement of epinephrine-induced lipolysis by T_3 in vitro is evident in 15–30 min (20–22). Furthermore, in the morphinized dog receiving a continuous infusion of isoproterenol, small doses of T_3 have been shown to increase the maximum rate of left ventricular tension development within 16 min, though similar doses of T_3 in the absence of isoproterenol infusion were without effect.[3] Both this interaction in the heart and that in adipose tissue (23), like the converse interaction that we now describe, apparently involve the beta-receptor, but little further is known of their mechanism.

A greater number of studies have been undertaken in efforts to explain the manner in which thyroid hormones act to enhance certain responses to adrenergic agents, but the ultimate mechanism or mechanisms remain uncertain, and data are contradictory in some respects. A review of the voluminous literature related to this topic is clearly beyond the scope of this report and, in any event, would not necessarily apply to the converse effect that we have seen in the thymocyte. Because hypothyroidism decreases and hyperthyroidism increases the increase in cyclic AMP induced by epinephrine in adipose tissue in vitro (24), and because dibutyryl cyclic AMP increases the accumulation of CLE by thymocytes in vitro (13), it might be thought that a greater cellular accumulation of cyclic AMP in the presence of T_3 and epinephrine than of either alone might explain these findings. This is unlikely, however, because dibutyryl cyclic AMP increases the accumulation of alpha-aminoisobutyric acid by increasing influx (13), whereas the potentiating action of epinephrine on CLE accumulation was demonstrably a result of an increase in the inhibition of CLE efflux that T_3 produces.

A central question concerning these observations relates to their physiological relevance. This question arises because to demonstrate an increased accumulation of CLE by rat thymocytes in vitro, either high concentrations of T_3 must be added directly (1 μM) or a large dose of T_3 (20 μg/100 g body wt) must be given to rats before thymocyte isolation. Recently, however, we have been able to define conditions in which the T_3-responsiveness of the thymocyte with respect to CLE accumulation is greatly increased. In thymocytes isolated from adrenalectomized rats, enhancement of CLE accumulation by T_3 in vitro is produced by concentrations as low as 0.1 nM, and this effect of adrenalectomy is abolished by pretreatment of the donor animals with physiological replacement doses of glucocorticoid.[4] Moreover, in the adrenalectomized rat, the quantity of T_3 by single intravenous injection that is required to increase accumulation of CLE by subsequently isolated thymocytes is as low as the daily physiological replacement dose, 0.5 μg/100 g body wt. Such sensitization of thymocytes from adrenalectomized animals to the effect of T_3 is consonant with the well-documented in vivo antagonism between physiological quantities of thyroid hormones and glucocorticoids, as well as their contrary independent effects, with respect to the growth and function of the lymphoid tissue (25–27). Such consonance suggests, in turn, that the effect of T_3 on amino acid accumulation that we are studying in vitro has its counterpart under physiologic circumstances in vivo. Additional evidence to this point is provided by the findings of Daniel et al. (28), who report that after intravenous administration of leucine, valine, and lysine, accumulation of the free forms of these amino acids by brain and skeletal muscle is far lower in hypothyroid than in normal rats.

High concentrations of thyroid hormones are often, though not invariably (29–31), required in vitro to produce effects that appear to have a physiological counterpart in vivo. For example, physiologic concentrations of thyroid hormone clearly modulate the lipolytic response of the rat to epinephrine because hypothyroidism greatly damps this response (24), but concentrations of T_3 required to enhance epinephrine-

[3] G. S. Kurland. Personal communication.

[4] Etzkorn, J. R., P. Hopkins, D. Gluckin, J. Gray, I. D. Goldfine, and S. H. Ingbar. 1979. Enhancement of glucocorticoid deficiency of the increase in cycloleucine accumulation induced in rat thymocytes by triiodothyronine. Manuscript submitted for publication.

stimulated lipolysis by rat adipose tissue in vitro are the same as or higher than the high concentrations that we have employed, 0.1–1.0 μM (20–22). Similarly, hypothyroidism greatly damps a variety of cardiovascular responses to catecholamines (1–3), but in the fetal rat heart in organ culture, for example, enhancement of the contractile rate requires T_3 concentrations of 0.1–10 μM (32). Previous authors have discussed the problems posed by, and possible explanations for, this discrepancy between in vivo and in vitro responses (14, 20).

A further question concerning these observations is their possible implications with respect to the mechanism of action of the thyroid hormones. Neither the stimulatory effect of T_3 on CLE accumulation nor the potentiation of this effect by epinephrine can be ascribed to nonspecific cytotoxicity of these agents. Thus, as judged from the ability of cells to exclude trypan blue, the viability of thymocytes was neither enhanced nor diminished by T_3, either alone or in combination with epinephrine. Neither can a more subtle cytotoxic effect of T_3 and epinephrine leading to an increase in cell water and in CLE diffusion space explain the stimulation of CLE accumulation because, as judged from studies with [^{14}C]urea, total cell water in thymocyte suspensions was unaffected by T_3 and epinephrine, either alone or in combination. Hence, more specific fundamental effects seem likely.

The stimulatory effects of T_3 on amino acid accumulation by chick embryonic bone and by thymocytes (12, 14), as well as the effect of T_3 to increase 2-deoxy-D-glucose uptake in both chick embryo myocardium (15) and rat thymocytes (16), like the effect of T_3 to enhance epinephrine-stimulated lipolysis (17), are all independent of new protein synthesis. The same is true of the potentiating action of epinephrine on the stimulation of CLE accumulation in thymocytes by T_3 that we have herein described. This, as well as the promptness of these effects, the fact that they relate to substrate influx or efflux, and the fact of their interaction with catecholamines, whose actions are thought to be mediated at the cell membrane, suggest that these actions of T_3 in vitro also occur at the cell surface. The likelihood that this is the case is increased, it would appear, by the recent demonstration of high affinity, limited capacity binding sites for T_3 in plasma membranes derived from rat liver (33).

If it be granted that these in vitro effects of T_3 have relevance to the physiologic actions of T_3 in vivo, then we would suggest that thyroid hormones act at the nuclear level to alter gene expression, probably act directly on the mitochondrion, and act at the cell membrane as well. In this light, thyroid hormones would be seen to act at multiple loci to induce a coordinated response in which increased availability of substrate proceeds hand in hand with increased energy metabolism and accelerated synthesis of specific proteins.

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