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Joseph Segal, Sidney H. Ingbar

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Research Article

Isolated rat thymocytes were preincubated with various catecholamines, alone and together with 3,5,3′-triiodothyronine (T_3), and the accumulation of the glucose analogues, 2-deoxy-d-glucose (2-DG) and 3-*O*-methylglucose (3-*O*-MG), was then measured. Epinephrine induced a time- and dose-dependent increase in the 15-min accumulation of 2-DG; at a concentration of 100 μM epinephrine, the effect was evident after a preincubation period of only 5 min. The lowest concentration of epinephrine at which a significant effect was evident was 1 μM. Epinephrine also produced a dose-dependent increase in the accumulation of 3-*O*-MG, and the lowest concentration at which a significant effect was evident was again 1 μM. Isoproterenol, a β-adrenergic agonist, like epinephrine, increased the accumulation of 2-DG, whereas the α-agonist, phenylephrine, had no effect. The response to epinephrine was inhibited by the β-antagonist, alprenolol, but the α-antagonist, phentolamine, had no effect. As previously demonstrated, T_3 increased 2-DG accumulation, and like epinephrine, its effect was blocked by alprenolol. Neither T_3 (0.1 nM) nor epinephrine (0.1 μM) had any effect when acting alone, but when added together at these concentrations, they significantly increased the accumulation of both 2-DG and 3-*O*-MG. Neither T_3 with isoproterenol nor T_3 with phenylephrine produced a comparable synergistic effect. But T_3 (0.1 nM) acting with isoproterenol (0.1 μM) and phenylephrine (0.1 μM) together, synergistically increased 2-DG accumulation. In addition, the α-antagonist, phentolamine, […]

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JOSEPH SEGAL and SIDNEY H. INGBAR, *Thorndike Laboratory of Harvard Medical School and Department of Medicine, Beth Israel Hospital, Boston, Massachusetts 02215*

ABSTRACT Isolated rat thymocytes were preincubated with various catecholamines, alone and together with 3,5,3′-triiodothyronine (T_3), and the accumulation of the glucose analogues, 2-deoxy-D-glucose (2-DG) and 3-*O*-methylglucose (3-*O*-MG), was then measured. Epinephrine induced a time- and dose-dependent increase in the 15-min accumulation of 2-DG; at a concentration of 100 μM epinephrine, the effect was evident after a preincubation period of only 5 min. The lowest concentration of epinephrine at which a significant effect was evident was 1 μM. Epinephrine also produced a dose-dependent increase in the accumulation of 3-*O*-MG, and the lowest concentration at which a significant effect was evident was again 1 μM. Isoproterenol, a β-adrenergic agonist, like epinephrine, increased the accumulation of 2-DG, whereas the α-agonist, phenylephrine, had no effect. The response to epinephrine was inhibited by the β-antagonist, alprenolol, but the α-antagonist, phentolamine, had no effect. As previously demonstrated, T_3 increased 2-DG accumulation, and like epinephrine, its effect was blocked by alprenolol. Neither T_3 (0.1 nM) nor epinephrine (0.1 μM) had any effect when acting alone, but when added together at these concentrations, they significantly increased the accumulation of both 2-DG and 3-*O*-MG. Neither T_3 with isoproterenol nor T_3 with phenylephrine produced a comparable synergistic effect. But T_3 (0.1 nM) acting with isoproterenol (0.1 μM) and phenylephrine (0.1 μM) together, synergistically increased 2-DG accumulation. In addition, the α-antagonist, phentolamine, which alone had no effect, inhibited the synergistic effect induced by T_3 and epinephrine. The effects of epinephrine and T_3 alone, as well as their combined synergistic effect on 2-DG accumulation, were not blocked by the inhibitor of protein synthesis, puromycin.

From these results we conclude the following: (*a*) the stimulatory effect of the catecholamines on the accumulation of 2-DG and 3-*O*-MG reflects an action at the β-receptor; (*b*) the synergistic interaction between T_3 and epinephrine requires the participation of both β- and α-adrenergic components; (*c*) T_3 and epinephrine act on 2-DG and 3-*O*-MG accumulation through a common mechanism or inter-related mechanisms, probably mediated at the β-adrenergic site; and (*d*) these effects of T_3 and epinephrine, alone and together, are independent of new protein synthesis. These results suggest that, with respect to the response we are describing, T_3 and epinephrine do not act on nuclear mechanisms, but may act instead at the level of the plasma membrane.

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INTRODUCTION

The primary mechanism of action of the thyroid hormones remains uncertain, despite extensive interest in this question. In previous studies we have presented evidence suggesting that, in addition to the actions on the nucleus and mitochondrion postulated by others (1–5), a primary action of the hormones is also exerted at the level of the plasma membrane. Part of this evidence is the finding that in the isolated rat thymocyte in vitro, 3,5,3′-triiodothyronine (T_3)[1] promptly stimulates both the accumulation of certain amino acids (6, 7) and the inward transport of glucose analogues (8), responses that are independent of new protein synthesis. An unusual feature of the effect of T_3 on amino acid accumulation is the fact that β-adrenergic agonists, which themselves have no effect on amino acid accumulation in this system, promptly synergize the stimulatory effect of T_3 (6). This finding seemed to

[1] *Abbreviations used in this paper*: 2-DG, 2-deoxy-D-glucose; ^{3}H-2-DG, D-[2-*deoxy*-^{3}H]glucose; 3-*O*-MG, 3-*O*-methylglucose; ^{3}H-3-*O*-MG, D-[3-*O*-^{3}H]methylglucose; T_3, 3,5,3′-triiodothyronine.

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point further toward an action mediated at the plasma membrane level. Consequently, we undertook the present studies to ascertain whether a similar interaction between T_3 and catecholamines takes place in respect to sugar transport. The data indicate that an analogous, but not identical, synergism does occur.

METHODS

Thymocytes were isolated from weanling female CD rats (25–30 d old, purchased from Charles River Breeding Laboratories, Wilmington, Mass.) by the method previously described by Goldfine et al. (9) with modifications previously employed in this laboratory (8). In short, rats were sacrificed by cervical dislocation, exsanguinated, and their thymus glands were quickly removed into ice cold Krebs-Ringer-Tris buffer, pH 7.5 (20 mM Tris-HCL, 5 mM Tris-base, 120 mM NaCl, 1 mM $CaCl_2$, 1.5 mM NaH_2PO_4, 2.5 mM MgCl, and 15 mM Hepes). The glands were washed with the cold buffer and gently teased with forceps. The free cells were filtered through nylon mesh to remove tissue debris and were then centrifuged at 4°C for 5 min at 300 *g*. Cells were suspended in buffer to a final concentration of 45×10^6 cells/ml and were allowed to equilibrate for an initial 30-min period in a metabolic shaker at 37°C under room air. Thereafter, they were preincubated for varying periods with the various agents to be tested, as indicated below. In most experiments, D-[2-*deoxy*-^{3}H]glucose (^{3}H-2-DG; 3 μCi/ml, 8.26 Ci/mmol sp act)[2] was then added and its accumulation in the cells was measured 15 min later. In some experiments, D-[3-*O*-*methyl*-^{3}H]glucose (^{3}H-3-*O*-MG; 3 μCi/ml, 80.8 Ci/mmol sp act), rather than ^{3}H-2-DG, was added and its accumulation in the cells measured 60 s later. As in previous studies, cells in separate vessels were incubated with [^{3}H]mannitol, as a marker of extracellular fluid volume (8).

Measurement of ^{3}H-2-DG and ^{3}H-3-O-MG uptake. At the end of the incubation, 200-μl aliquots of the cell suspensions were quickly removed into microtubes (Beckman Instruments, Inc., Fullerton, Calif.) and centrifuged at 10,000 rpm (Beckman microfuge) for 30 s. The cell pellet was taken up in toluene-Triton X-100 (Rohm and Haas Co., Philadelphia, Pa.) scintillation fluid, and its ^{3}H content was measured in a liquid scintillation counter.

Effect of puromycin. In experiments designed to assess the role of new protein synthesis in the responses observed, thymocytes were preincubated for 10 min without or with a concentration of puromycin (100 μg/ml) previously shown to produce >95% inhibition of [^{3}H]leucine incorporation by rat thymocytes in vitro (8). Stimulatory agents were then added for an additional 60 min, and the 15-min uptake of ^{3}H-2-DG was then measured.

Cell viability. Cell viability was evaluated by microscopically assessing the ability of the cells to exclude trypan blue (final concentration, 0.3 g/dl) added at the end of the various incubation periods.

Statistics. Statistical methods employed were the Dunnett multiple comparison procedure for comparing the results in several treatments with those obtained in a control group or analysis of variance followed by the Newman-Keuls multiple range test for comparing values obtained among multiple groups (10).

RESULTS

Effect of epinephrine. Initial experiments were conducted to ascertain the effects of varying concentrations of epinephrine, preincubated with thymocytes for varying periods, upon 2-DG accumulation. For periods of preincubation as long as 30 min, epinephrine at concentrations of 1, 10, and 100 μM produced a dose-related, temporally progressive enhancement of 2-DG accumulation, which was a statistically significant effect at 20, 10, and 5 min of preincubation, respectively (Fig. 1). With 60-min periods of preincubation, still further enhancement of 2-DG accumulation continued in vessels that contained 1 and 10 μM epinephrine, but a dramatic decrease in 2-DG accumulation was seen in those vessels with 100 μM epinephrine. Studies with trypan blue indicated that cell viability in thymo-

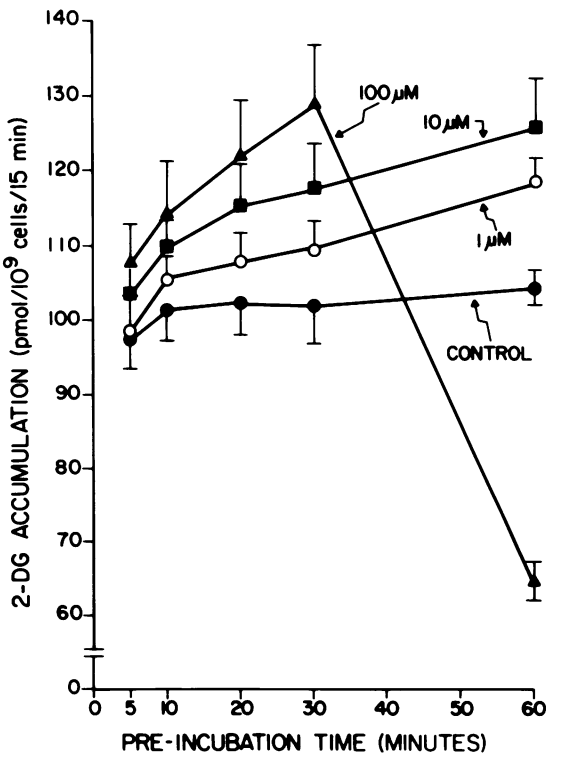

FIGURE 1 Time-course of the effect of epinephrine on 2-DG accumulation by rat thymocytes in vitro. Cells were preincubated at 37°C for the indicated times in the presence of the concentrations of epinephrine shown. ^{3}H-2-DG was then added and its intracellular accumulation determined 15 min later. Values shown are the mean±SD of mean values obtained in four separate experiments in which quadruplicate vessels were studied for each point. Significant increases above control values ($P < 0.01$) were obtained at 5 min with 100 μM epinephrine, at 10 min with 10 μM epinephrine, and at 20 min with 1 μM epinephrine.

[2] D-[2-*deoxy*-^{3}H]glucose and D-[3-*O*-^{3}H]methylglucose were purchased from New England Nuclear (Boston, Mass.). L-epinephrine, L-isoproterenol, DL-propranolol, L-alprenolol, L-phenylephrine, L-3,5,3′-triiodothyronine, and puromycin were purchased from Sigma Chemical Co. (St. Louis, Mo.). L-phentolamine was a gift from CIBA-Geigy Corp., Pharmaceuticals Division (Summit, N. J.).

cytes that were preincubated with 100 μM epinephrine for 60 min had decreased to 60% but had remained unchanged at ~95% in all other vessels, including controls.

In view of the foregoing findings, the dose-response relationship for the effect of epinephrine was assessed by using a 20-min preincubation period. Under these conditions, a clear log dose-response relationship was evident (Fig. 2). The lowest concentration of epinephrine that produced significant stimulation was 1 μM, whereas the greatest effect was produced by the highest concentration tested (1 mM). Cell viability was unchanged (93–95%) in all specimens.

At the concentrations of epinephrine routinely employed (≤10 μM), cell viability was retained during 60-min incubations and the effect on 2-DG uptake was greater than at earlier time periods (Fig. 1). In view of that fact, and because the effect of T_3 is also maximum at 60 min (8), the 60-min time period was employed in most subsequent incubations.

Effect of isoproterenol. To ascertain the extent to which the effects of epinephrine would be mimicked by the β-agonist, isoproterenol, varying concentrations of isoproterenol were preincubated with thymocytes for 60 min. Experiments in which only isoproterenol was studied revealed that the quantitative response was almost exactly the same as that to epinephrine. This was also the case when the two agonists were directly compared within the same experiments (Fig. 3). As with epinephrine, a decrease in 2-DG accumulation was produced by 0.1 and 1 mM isoproterenol, which was also associated with a decrease in cell viability to ~60 and 20%, respectively.

Effect of phenylephrine. At concentrations ranging between 10 nM and 1 mM, the α-agonist, phenylephrine, had no effect on either 2-DG accumulation or cell viability when preincubated with thymocytes for 60 min (data not shown).

Effect of phentolamine. At concentrations ranging between 10 nM and 1 mM, 60 min preincubation with the α-antagonist, phentolamine, had no effect on 2-DG accumulation or cell viability. Similarly, phentolamine, at concentrations as high as 1 mM, had no significant effect on the stimulatory responses to 1 and 10 μM epinephrine (data not shown).

Effect of propranolol. In individual experiments, propranolol, generally considered a β-antagonist, proved to be a weak agonist with respect to 2-DG accumulation, there being no effect at 1 μM, but stimulatory effects at 10 and 100 μM propranolol. These effects were reproduced in experiments shown in Fig. 3, in which the effects of epinephrine, isoproterenol, and propranolol

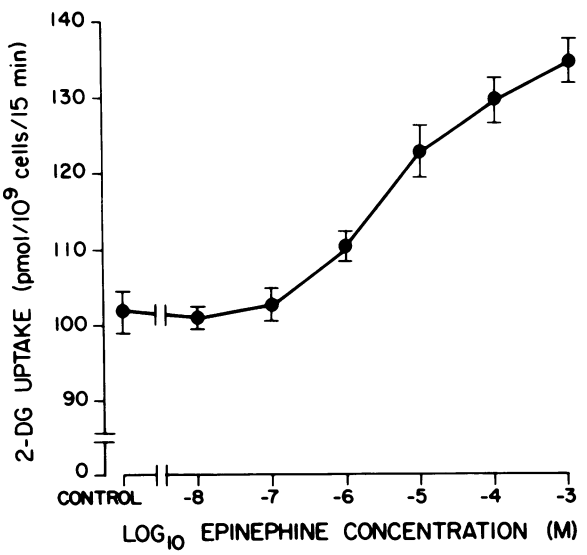

FIGURE 2 The effect of varying concentrations of epinephrine on 2-DG accumulation by rat thymocytes in vitro. Cells were preincubated with the indicated concentrations of epinephrine for 20 min. ^{3}H-2-DG was then added and its intracellular accumulation measured 15 min later. Significant stimulation ($P < 0.01$) was evident at epinephrine concentrations of 1 μM and higher. Values shown are the mean±SD of values obtained in three separate experiments (quadruplicate vessels for each point).

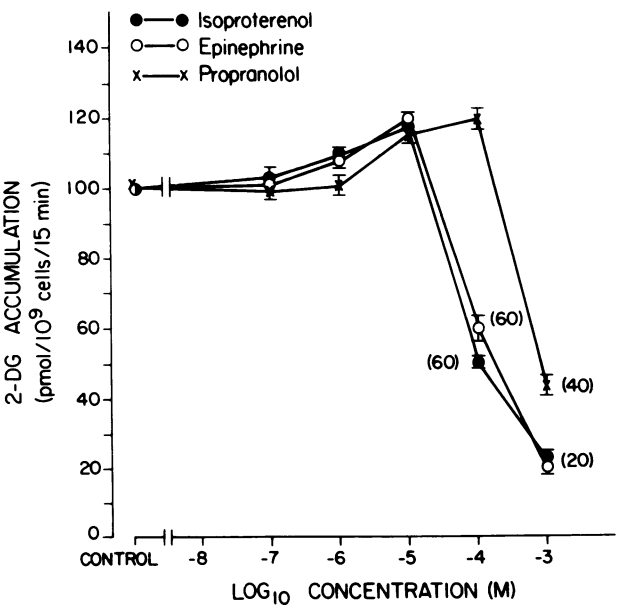

FIGURE 3 The effect of varying concentrations of epinephrine, isoproterenol, and propranolol on 2-DG accumulation by rat thymocytes in vitro. Cells were preincubated with varying concentrations of the several agents, as shown. ^{3}H-2-DG was then added and its intracellular accumulation measured 15 min later. Values shown are the mean±SD of mean values obtained in four experiments. Differences from control were statistically significant at 1 μM and above for epinephrine and isoproterenol, and at 10 μM and above for propranolol. Values shown in parentheses indicate percent cell viability at the corresponding points. Cell viability at all other points was 93–95%.

TABLE I

Effects of Propranolol and Epinephrine, Alone and Together, on the Accumulation of 2-DG by Rat Thymocytes In Vitro

		2-DG accumulation				
		Propranolol				
Epinephrine	μM, ...	0	0.01	0.1	1.0	10
μM		% control				
0		100.0*	100.0±1.1	100.0±3.2	100.7±2.7	115.3±2.8§
1		105.4±1.3‡	105.5±1.7‡	106.3±2.9‡	106.5±1.0‡	115.1±4.2§
10		114.6±2.5§	114.7±1.4§	116.9±2.5§	115.4±1.9§	116.3±5.4§

* Values shown are mean±SD of those obtained in four separate experiments, expressed as a percentage of the hormone-free control. Mean control value, 114.0±2.1 pmol/10^9 cells per 15 min.

‡ $P < 0.05$.

§ $P < 0.01$.

were directly compared. At very high concentrations (1 mM), propranolol also decreased 2-DG accumulation, an effect associated with a decrease in cell viability to ~40%. At concentrations between 10 nM and 1 μM, propranolol did not alter the stimulatory responses of 2-DG accumulation to 1 and 10 μM epinephrine. Propranolol at 10 μM was itself stimulatory; in combination with stimulatory concentrations of epinephrine, 2-DG accumulation was equal only to that produced by propranolol alone (Table I).

Effect of alprenolol. When studied alone, the β-antagonist alprenolol had no effect on 2-DG accumulation at concentrations ranging between 10 nM and 10 μM. However, significant stimulation of 2-DG accumulation (10.5%) was produced by 0.1 mM alprenolol and still greater stimulation (15.9%) by 1 mM alprenolol (Fig. 4). Thus, like propranolol, alprenolol proved to behave as a weak agonist with respect to 2-DG accumulation. However, unlike propranolol, 1 mM alprenolol had no adverse effect on cell viability.

Effect of alprenolol with epinephrine. At concentrations that were without effect on 2-DG accumulation when added alone (1–10 μM), alprenolol completely inhibited the stimulatory response to 1 μM epinephrine and partially but significantly, inhibited the stimulatory response to 10 μM epinephrine (Table II).

Role of oxidation of epinephrine. To explore the possible involvement of the oxidation of epinephrine in its effect on 2-DG accumulation, studies were performed with the monoamine oxidase inhibitor, pargyline. When added alone in concentrations between 0.1 and 10 μM, pargyline had no effect. Nor did those concentrations of pargyline influence the response to 1 and 10 μM epinephrine (data not shown).

Spectrophotometric analysis (11) revealed that after as much as 120 min of incubation of thymocytes with 10 μM epinephrine, concentrations of adrenochrome were below the limit of detectability (10 nM).

Interactions between adrenergic agents and T_3. Because of the fact that, like epinephrine, T_3 is capable of enhancing the uptake of 2-DG by rat thymocytes in vitro (8), possible interactions between T_3 and the adrenergic agents were explored. In experiments in which thymocytes were preincubated for 60 min with varying concentrations of T_3, with or without 0.1 μM

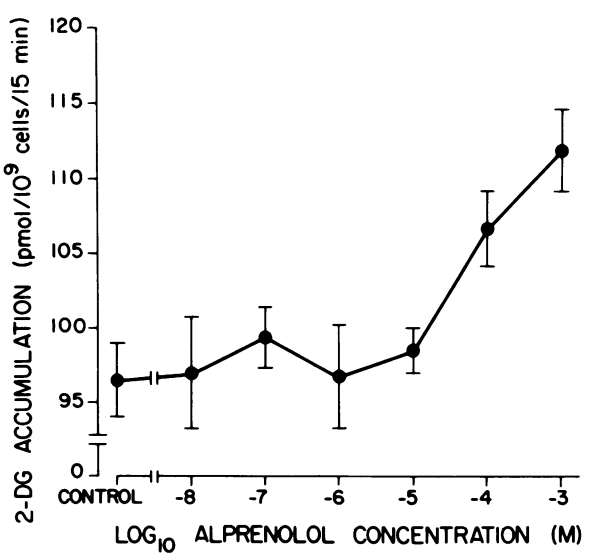

FIGURE 4 The effect of varying concentrations of alprenolol on the 15-min accumulation of 2-DG by rat thymocytes in vitro. Cells preincubated with the indicated concentration of alprenolol for 60 min before the addition of ^{3}H-2-DG. Values shown are mean±SD of mean values obtained in three separate experiments (quadruplicate vessels). Significant increases in 2-DG accumulation seen at 0.1 mM ($P < 0.05$) and 1 mM ($P < 0.01$) alprenolol.

TABLE II

Alprenolol Inhibition of Epinephrine—Stimulated Accumulation of 2-DG by Rat Thymocytes In Vitro

Epinephrine	Alprenolol	2-DG accumulation*	Effect	*P* values vs. control
μM	μM	*pmol/10⁸ cells/15 min*	*% change*	
—	—	106.4±3.6	—	—
1	—	113.3±3.8	+6.5	<0.05
10	—	136.2±3.8	+28.0	<0.01
—	1	108.2±2.2	+1.7	NS
—	10	110.3±3.8	+3.7	NS
1	1	106.5±3.7	+0.1‡	NS
1	10	107.0±2.5	+0.6‡	NS
10	1	129.8±2.8	+22.0§	<0.01
10	10	130.3±3.4	+22.5§	<0.01

* Values shown are mean±SD of mean values obtained in four different experiments.
‡ Significantly ($P < 0.05$) lower than 1 μM epinephrine alone.
§ Significantly ($P < 0.05$) lower than 10 μM epinephrine alone.

epinephrine, no effect on 2-DG accumulation was produced by epinephrine alone or by 10 or 100 pM T_3. However, when 0.1 μM epinephrine and 100 pM T_3 were added together, a clear stimulation of 2-DG accumulation was seen. T_3, at a concentration of 1 nM, was itself significantly stimulatory and a significantly greater stimulation was evident when the otherwise ineffective concentration of epinephrine was added. Thus, the data clearly indicated a synergistic interaction between the two agents (Fig. 5).

In contrast to the findings with epinephrine, no evidence of a synergistic interaction between T_3 and isoproterenol could be obtained. Thus, no combination of individually ineffective concentrations of the two agents produced a significant stimulatory effect. Furthermore, when effective concentrations of one or both were employed, the percent increment in 2-DG accumulation above basal values did not significantly exceed the sum of the percent increments produced by each agent alone (data not shown).

In combinations of T_3 with a wide range of concentrations of phenylephrine (0.1–10 μM), the 2-DG accumulation was equal to that seen in the presence of T_3 alone (data not shown).

Because epinephrine is known to be an agaonist for both α- and β-receptors, and because neither phenylephrine, an α-agonist, nor isoproterenol, a β-agonist, interacted synergistically with T_3 as epinephrine did, experiments were conducted in which the effects of varying mixtures of T_3 with phenylephrine and isoproterenol were studied. Isoproterenol at a concentration of 0.1 μM together with phenylephrine at concentrations of 1 and 0.1 μM had no effect on 2-DG accumulation in the absence of T_3. However, in combination

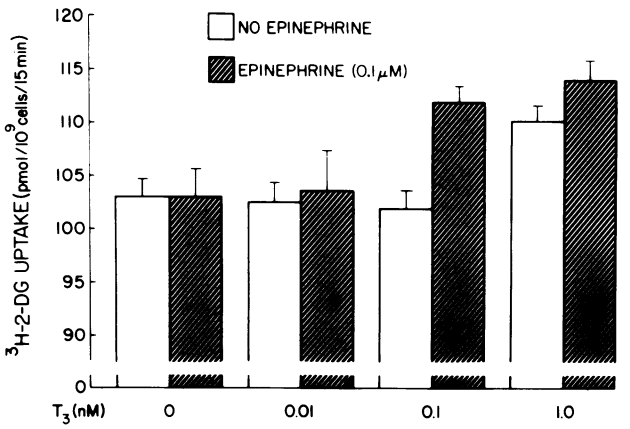

FIGURE 5 Synergistic effects of epinephrine and T_3 to stimulate the 15-min accumulation of 2-DG by rat thymocytes in vitro. Cells preincubated with the indicated concentrations of T_3 and epinephrine for 60 min before the addition of ^{3}H-2-DG. Results shown are mean±SD of mean values obtained in at least four experiments for each experimental pair. As judged from the Newman-Keuls multiple range test (10), values in the presence of epinephrine were significantly higher than with T_3 alone in the case of T_3 concentrations of 0.1 nM ($P < 0.01$, $n = 12$) and 1.0 nM ($P < 0.05$, $n = 6$).

with an ineffective concentration of T_3 (0.1 nM), significant stimulation of 2-DG accumulation became evident (Fig. 6).

From the foregoing findings, it appeared that both α- and β-adrenergic stimulation were required for the

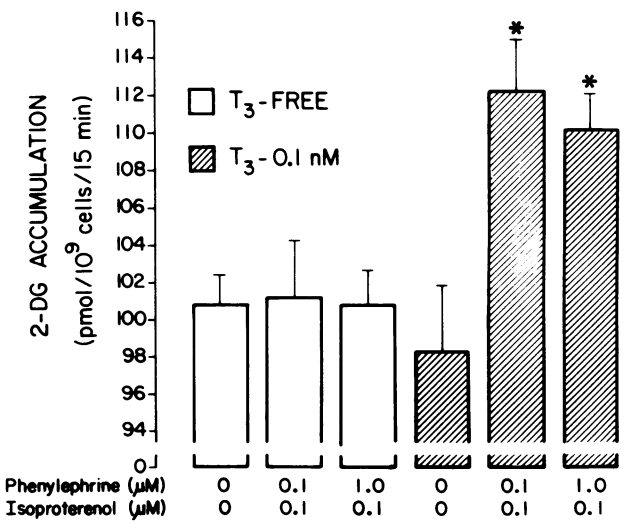

FIGURE 6 Synergistic interaction of T_3 with phenylephrine plus isoproterenol to increase the accumulation of 2-DG by rat thymocytes in vitro. Cells preincubated with the indicated concentrations of experimental agents for 60 min. ^{3}H-2-DG was then added and its intracellular accumulation measured 15 min later. Results shown are mean±SD of mean values obtained in five experiments (quadruplicate vessels). Values shown by asterisks are significantly different from all others ($P < 0.01$), but not from each other.

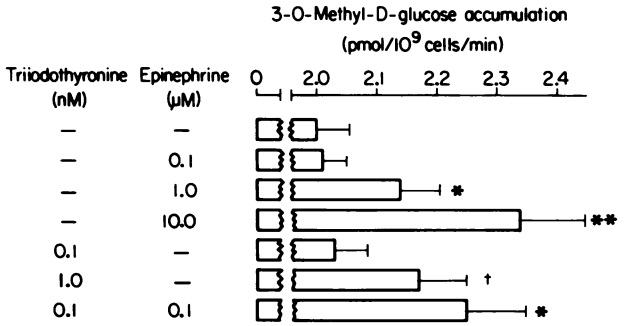

FIGURE 7 Synergistic effects of epinephrine and T_3 on the accumulation of 3-O-methylglucose by rat thymocytes in vitro. Cells were preincubated with indicated concentrations of agents for 60 min. ^{3}H-3-*O*-MG was then added and its intracellular accumulation measured 60 s later. Values shown represent mean±SD of mean values obtained in six experiments (triplicate vessels). Asterisks indicate significant difference from value in corresponding group containing no epinephrine. *-$P < 0.05$; **-$P < 0.01$; † indicates significant difference from hormone-free control ($P < 0.05$).

synergistic interaction of catecholamines and T_3. To obtain further evidence to this point, experiments were conducted to determine the effect of α-adrenergic blockade on the synergistic interaction between T_3 and epinephrine. Although, as already described, phentolamine (1 and 10 μM) had no effect on 2-DG accumulation when added alone, and did not significantly modify the effects of T_3 or epinephrine added individually, phentolamine at these concentrations completely blocked the synergistic interaction evident when T_3 at 0.1 nM and epinephrine (0.1 μM) were added together (Table III).

Effect of puromycin. Preincubation with puromycin decreased the basal uptake of ^{3}H-2-DG by ~20%, but did not significantly affect the percent increase in ^{3}H-2-DG uptake produced by epinephrine, either alone or acting synergistically with T_3. In four experiments, epinephrine (10 μM) increased the ^{3}H-2-DG uptake by an average of 17.2±1.4% in control cells and 16.8±1.2% in cells pretreated with puromycin. Similarly, in four experiments, the combination of T_3 (0.1 nM) and epinephrine (0.1 μM) increased ^{3}H-2-DG uptake by 10.7 ±1.1% in control cells and by 12.4±0.8% in cells exposed to puromycin.

Effect of adrenergic antagonists on the response to T_3. Phentolamine, at concentrations of 1 and 10 μM, did not significantly influence stimulatory responses elicited by 1 and 10 nM T_3. Alprenolol, on the other hand, at concentrations previously shown to be ineffective when added alone (1 and 10 μM), partially inhibited or completely blocked the stimulatory responses to 1 or 10 nM T_3 (Table IV).

Studies with 3-O-MG. To ascertain whether the effect of epinephrine alone and its synergism with T_3 to stimulate the accumulation of 2-DG requires phosphorylation of the sugar, studies were performed with 3-*O*-MG, which uses the same membrane transport mechanism as 2-DG does, but, unlike 2-DG, does not undergo phosphorylation. Epinephrine alone at concentrations of 1 and 10 μM significantly increased the 1-min accumulation of 3-*O*-MG, but 0.1 μM epinephrine did not (Fig. 7). Stimulatory concentrations of epinephrine were the same as those required in the case of 2-DG accumulation. As previously reported, T_3

TABLE III
Phentolamine Inhibition of Synergistic Effects of T_3 and Epinephrine on 2-DG Accumulation by Rat Thymocytes

T_3	Epinephrine	Phentolamine	2-DG accumulation*	Effect	*P* values vs. control
nM	*μM*	*μM*	*pmol/10⁹ cells/15 min*	*% change*	
—	—	—	108.0±2.4	—	—
0.1	—	—	107.1±3.0	−0.8	NS
—	0.1	—	109.2±2.5	+1.1	NS
—	—	1.0	109.1±3.9	+1.0	NS
—	—	10.0	108.3±2.4	+0.3	NS
0.1	0.1	—	122.7±2.2	+13.6	<0.01
0.1	0.1	1.0	107.0±4.6	−0.9‡	NS
0.1	0.1	10.0	110.1±2.8	+1.9‡	NS

* Values shown are mean±SD of those obtained in five separate experiments.
‡ Significantly ($P < 0.01$) different from the value obtained by 0.1 nM T_3 and 0.1 μM epinephrine in the absence of phentolamine.

TABLE IV
Effect of T_3 on 2-DG Uptake in the Presence and Absence of Alprenolol and Phentolamine

T_3	Alprenolol	Phentolamine	2-DG accumulation*	Effect	*P* value vs. control
nM	*μM*	*μM*	*pmol/10⁹ cells/15 min*	*% change*	
—	—	—	97.7±2.8	—	—
1.0	—	—	102.8±2.3	+5.2	<0.05
10.0	—	—	107.6±2.7	+10.1	<0.01
—	10.0	—	98.1±3.4	+0.4	NS
—	—	10.0	96.9±4.0	−0.8	NS
1.0	1.0	—	99.1±2.7	+1.4	NS
10.0	1.0	—	104.1±1.8	+6.6	<0.05
1.0	10.0	—	99.4±2.2	+1.7	NS
10.0	10.0	—	101.4±2.1‡	+3.8	NS
1.0	—	1.0	103.0±1.4	+6.0	<0.05
10.0	—	1.0	109.2±3.6	+11.8	<0.01
1.0	—	10.0	103.9±3.2	+6.3	<0.05
10.0	—	10.0	108.0±2.9	+10.5	<0.01

* Values shown are mean±SD of those obtained in four separate experiments.
‡ Significantly ($P < 0.025$) different from 10 nM T_3 alone.

alone at a concentration of 1 nM significantly stimulated 3-*O*-MG accumulation, but 0.1 nM T_3 was ineffective. When the ineffective concentrations of T_3 (0.1 nM) and epinephrine (0.1 μM) were added together, however, a significant stimulation of 3-*O*-MG uptake was seen.

DISCUSSION

We have previously presented evidence that various in vitro responses to T_3 are mediated by extranuclear mechanisms, possibly at the level of the cell membrane (6–8). Evidence in support of this view was the ability of T_3 in vitro to promptly stimulate, in isolated rat thymocytes, the accumulation of the amino acid analogues, α-amino isobutyric acid, and cycloleucine, as well as the inward transport of the glucose analogues, 2-DG and 3-*O*-MG. In the case of cycloleucine accumulation, β-adrenergic agonists, though themselves without effect, synergistically increased the stimulatory response to T_3 (6). In these studies, we have examined the interaction between T_3 and the adrenergic system with respect to sugar transport. Three major aspects were examined: the effect of adrenergic agents alone; the interactions of T_3 added alone with the adrenergic system; and the interactions between T_3 and adrenergic agents when added together.

Effects of adrenergic agents. The data clearly indicate that certain adrenergic agents stimulate the 15-min accumulation of 2-DG in rat thymocytes by a mechanism mediated at the β-adrenergic receptor site. At the lowest significantly effective epinephrine concentration (1 μM), an effect was produced by 20 min of preincubation, but at higher, more effective concentrations shorter periods of preincubation were required (5 min in the case of 0.1 mM). The stimulatory effect of epinephrine was reproduced by the β-adrenergic agonist, isoproterenol, but not by the α-agonist, phenylephrine, and was not blocked by the α-adrenergic antagonist, phentolamine.

Data concerning the β-adrenergic antagonists, though more complex, also support the β-mediation of direct stimulatory effects on 2-DG accumulation. Both propranolol and alprenolol behave as weak agonists with respect to 2-DG accumulation. This was particularly true of propranolol, which, in high concentration (10 μM), was as effective as an equal concentration of epinephrine and isoproterenol. This obscured the interpretation of experiments in which epinephrine and propranolol were added together. However, in the case of the weaker agonist of 2-DG accumulation, alprenolol, it was possible to demonstrate that low concentrations (1 and 10 μM), which had no effect when added alone, partly or completely inhibited the stimulatory response to epinephrine.

These stimulatory effects of epinephrine and isoproterenol on 2-DG accumulation in rat thymocytes complement a lengthy list of effects of adrenergic agonists on sugar transport and metabolism in a variety of other tissues that have been reported by previous investigators. There appears to be little or no uniformity in the pattern of response, however, because both stimulatory and inhibitory effects have been described, and because the influence of the adrenergic antagonists on these effects have also been highly variable (12, see review).

Similarly, the foregoing agonistic effects of the classical β-antagonists on a β-mediated function, though unusual, are not without precedent. Thus, both epinephrine and propranolol independently stimulate the uptake of 3-*O*-MG by geese erythrocytes in vitro (13), and, in the isolated canine heart, the β-antagonists practolol, and pindolol both act like norepinephrine to enhance glucose uptake (14). Further, both the chronotropic and inotropic effects of isoproterenol are mimicked by practolol in the isolated rat artium (15). Similar responses have been seen in other systems (16, 17).

Interaction of T_3 alone with the adrenergic system. An unusual finding in the present study was the ability of the β-adrenergic antagonist, alprenolol, to block the stimulation of 2-DG accumulation produced by T_3 alone, this occurring in a system that should have been devoid of any endogenous catecholamine. The nature of the response to T_3 in the presence of propranolol could not be evaluated, because of propranolol's stronger independent agonistic effect; however, the α-adrenergic antagonist, phentolamine, had no effect on the stimulatory response to T_3.

This in vitro inhibitory effect of alprenolol on the response to T_3 alone is reminiscent of that reported by Popovic and co-workers (18), who demonstrated that thyroxine in vitro increases the erythroid colony forming activity of canine marrow cells and that this stimulation is inhibited by propranolol. Apart from these two responses, we know of no other system in which an effect of thyroid hormone that occurs in the absence of catecholamines is blocked by an adrenergic antagonist.

Interactions of T_3 and adrenergic agents. A major finding of this study, and further evidence of a relationship between the action of T_3 and the adrenergic system, is the demonstration of a prompt synergistic interaction between T_3 and epinephrine with regard to 2-DG accumulation. We find this interaction both unusual and unusually interesting in several respects. Thus, neither the β-agonist, isoproterenol, nor the α-agonist, phenylephrine, displayed a synergistic interaction with T_3 when added separately, though they did so when added together. This effect of the combined α- and β-agonists was similar to that of epinephrine, which is known to have both α- and β-agonistic activity. Furthermore, although phentolamine had no effect on the separate stimulatory effects of epinephrine and T_3, it inhibited the synergistic interaction between the two. From these findings, it appears that al-

though the independent effects of T_3 and of the catecholamines on 2-DG accumulation by the thymocyte are β-mediated, their synergistic stimulatory action requires participation of the alpha receptor.

Prompt synergistic interactions between thyroid hormones and catecholamines on defined metabolic responses, have been noted in other systems. For example, T_4 has been shown promptly to increase the epinephrine-induced release of α-amylase by rat-parotid gland in vitro (19). The best studied of such interactions are those in which T_3 enhances the in vitro lipolytic response to epinephrine in rat adipose tissue (20–22). These interactions differ, however, in several respects from the ones that we describe here. First, in the two foregoing systems, the action of epinephrine alone, like that we describe in thymocytes (6), is clearly β-mediated, but the thyroid hormones themselves are said to have no independent stimulatory effect. Second, with respect to these systems, there is no evidence that the participation of an α-component in the synergistic interaction between thyroid hormones and epinephrine is required.

Properties of the response to T_3 and adrenergic agents. The stimulatory effect of epinephrine on 2-DG accumulation was evidently not caused by metabolites of epinephrine generated during incubation, rather than by epinephrine itself. Pargyline, a monoamine oxidase inhibitor, did not alter the response to epinephrine, and detectable concentrations of adrenochrome (>10 nM) were not generated during incubations that contained epinephrine in concentrations as high as 10 μM.

New protein synthesis was not required for either the effect of epinephrine on 2-DG accumulation or the synergistic stimulatory interaction between epinephrine and T_3, because a puromycin concentration that greatly inhibits protein synthesis in this system (8) had no effect on either response.

We have earlier shown that T_3 enhances the accumulation of 2-DG in rat thymocytes by increasing the transport of the sugar without affecting its phosphorylation. This conclusion was supported by the finding that T_3 also enhanced the accumulation of 3-*O*-MG, which is transported into the cell by the same system as 2-DG, but is not phosphorylated (8). In the present experiments we again used 3-*O*-MG, this time to determine whether the stimulatory effect of epinephrine and its synergistic interaction with T_3 might reflect, not a stimulation of sugar transport, but solely an effect on the phosphorylation of 2-DG. This proved not to be the case, however, because both the independent effect of epinephrine and its synergistic interaction with T_3 in regard to 2-DG accumulation were paralleled by effects on 3-*O*-MG uptake.

General conclusions. From the data obtained in our present and previous studies of sugar metabolism in rat thymocytes (8), we are led to conclude that T_3 and epinephrine can independently act to stimulate the inward transport of sugars, at least in this cell, and that their independent actions are each mediated at the β-adrenergic receptor. When present together, their action is more complex, however. Under these conditions, they synergistically enhance inward transport of sugar, but the synergism now requires participation of an α-adrenergic component. Quite clearly, we are unable to explain this interesting feature of the response. However, because these actions and interactions are very prompt in onset and are not dependent upon new protein synthesis, we would suggest that their mediation is extranuclear, and probably takes place at the cell membrane. This view is consonant with our recent demonstration of saturable binding of both T_3 and epinephrine to preparations of purified plasma membranes of rat thymocytes.[3]

Superficially, the interaction between T_3 and catecholamines with respect to 2-DG accumulation in rat thymocytes resembles the interaction we have previously reported with respect to cycloleucine accumulation in the same cell (6). Both interactions are synergistic in nature and both appear to be mediated at an extranuclear site, in as much as neither is blocked by profound inhibition of protein synthesis. Nevertheless, the most intriguing aspect of the present findings, in our view, is the extent to which the properties of the T_3-catecholamine interaction with respect to the two metabolic responses differ. Thus, in the case of cycloleucine accumulation: T_3 alone is stimulatory, but the catecholamines alone have no effect; the response to T_3 is unaffected by either α- and β-adrenergic antagonists; and the synergistic interaction between T_3 and epinephrine is blocked by β-antagonists, but is unaffected by the α-antagonist phentolamine. We cannot explain these differences in the properties of two metabolic responses to the same stimuli within the same cell. It seems likely, however, that the explanation, when available, will help to elucidate the independent mechanisms of the actions of T_3 and the catecholamines in these systems, as well as the mechanisms of their interaction with one another.

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