LIPOLYTIC ACTION OF 3,3'5-TRIIODO-L-THYRONINE, A CYCLIC AMP PHOSPHODIESTERASE INHIBITOR

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The mechanism by which 3,3'5-triiodo-L-thyronine (T_3) increases the sensitivity of adipose tissue to epinephrine is unknown. The rate of lipolysis in adipose tissue from rats treated with T_3 is increased over control levels and the effect of epinephrine on release of free fatty acids and glycerol is greater in tissues from T_3-treated rats than from control tissues (Debons and Schwartz, 1961; Deykin and Vaughan, 1963). The effect of T_3 on epinephrine induced lipolysis is apparent as early as 3 hours after intravenous administration to thyroidectomized rats (Bray and Goodman, 1965).

Direct addition of low levels of T_3 ($2x10^{-6}M$) to rat epididymal fat pads incubated *in vitro* has been reported to have no effect on basal or epinephrine stimulated lipolysis (Debons and Schwartz, 1961). It has now been found that when rat epididymal lipocytes are exposed to higher levels of T_3 ($10^{-3}M$ - $10^{-4}M$) there is a marked stimulation of lipolysis measured by glycerol production. This is blocked by insulin and prostaglandin E_1 but not by the beta adrenergic blocking agent Kö 592. T_3 acts synergistically with low levels of norepinephrine and epinephrine in increasing both glycerol release and the intracellular content of adenosine 3'5'monophosphate (cyclic AMP) in rat epididymal fat tissue. T_3 is shown to be a competitive inhibitor of cyclic AMP phosphodiesterase, suggesting it exerts its lipolytic action by preventing the degradation of cyclic AMP.

MATERIALS AND METHODS

Male Charles River rats, 160-200 gm, given food and water *ad libitum* were

used for all these studies. T_3 (sodium salt) and related compounds were purchased from Mann Research Laboratories, cyclic AMP was purchased from Calbiochem, ATP-γ-P^{32} (spec. act. 800 mc/mmole) was obtained from the I.C.N. Corp., and H^3-cyclic AMP (spec. act. 1.0C/mmole) was obtained from Schwarz Bioresearch, Inc. Prostaglandin E_1 was a gift of Dr. S. Bergström (Karolinska Institute, Stockholm, Sweden) and Kö 592 (1-(3-methylphenoxy)-2-hydroxy-3-isopropylamino propane) was a gift of Dr. A. Engelhardt (C. H. Boehringer Sohn, Ingelheim, Germany). All other chemicals were commercially available.

For the in vitro lipolysis experiments isolated fat cells were prepared from rat epididymal fat pads by the method of Rodbell (1964). Fat cells were suspended in Krebs-Ringer-phosphate buffer (Cohen, 1957) containing 2% bovine plasma albumin fraction V (Armour) without glucose. 1 ml aliquots of the fat cell suspension were incubated 90 min at 37°. Each ml of suspension represented 0.05 gm of epididymal fat.

For experiments on the measurement of intracellular cyclic AMP levels, fat pads were prepared and incubated by methods similar to those described by Butcher et al. (1965). Cyclic AMP was separated from other materials by chromatography on Dowex-2 and assayed by modification of the method of Posner, J., et al. (1964). The conversion of phosphorylase b to phosphorylase a was carried out in the presence of ATP-γ-P^{32}. This reaction was stopped with TCA. The radioactivity incorporated into the precipitated P^{32}-labelled phosphorylase a was proportional to the amount of cyclic AMP present in the "activation step." As little as 5×10^{-11} moles cyclic AMP per gm of tissue (wet weight) could be detected by this procedure

Beef heart phosphodiesterase (20,000 x g supernatant fraction) was prepared by the method of Butcher and Sutherland (1962). The 0.6 ml assay mixture contained H^3-cyclic AMP (0.5μmole; 1.0μc) and the H^3-5'AMP formed was separated by paper chromatography in a solvent of 70 parts isopropanol - 20 parts 0.1M boric acid - 10 parts ammonia. When the enzyme was prepared from rat epididymal fat (Weiss, et al., 1966) a 0.3 ml assay mixture containing H^3-cyclic AMP (0.025μmoles 0.5μc) was used. Because two products were formed (5'AMP and adenosine) a differ-

ent solvent system was used for chromatography (30 parts 1M ammonium acetate pH 7.5 - 75 parts ethanol). The radioactivity in the areas on the chromatograms containing cyclic AMP, 5'AMP and adenosine was determined and the percent hydrolysis of cyclic AMP calculated.

T_3 and related compounds were dissolved in dimethylsulfoxide; the final dimethylsulfoxide concentration in the incubation mixtures was 1-3% (v/v).

RESULTS AND DISCUSSION

In table I the results of the lipolysis experiments are presented. 10^{-3} - 10^{-4}M T_3 produced up to a 7-fold stimulation of glycerol release. At 10^{-5}M a marked potentiation of norepinephrine lipolysis was observed suggesting that T_3 acts at a site other than the one affected by norepinephrine. That is, T_3 does not act directly on adenyl cyclase. Of the compounds structurally related to T_3 only 3,5-diiodo-L-thyronine produced similar results.

TABLE I

Effect of Norepinephrine, T_3 and Related Compounds on Glycerol Release in Isolated Fat Cells

Addition	Concentration (M)	Glycerol Release (mμmoles/ml/1.5 hr)± SE without norepinephrine	with 5 x 10^{-8}M DL norepinephrine
control		50±2	140±21
DL-thyroxin	10^{-3}-10^{-4}	60±13	156±7
T_3	10^{-3}	350±40	440±15
	10^{-4}	260±70	570±40
	10^{-5}	28±11	420±55
3,3',5-triiodo-D-thyronine	10^{-3}-10^{-4}	58±11	232±36
3,5-diiodo-L-thyronine	10^{-3}	345±50	556±43
	10^{-4}	222±22	480±10
	10^{-5}	45±11	335±33
L-thyronine	10^{-3}-10^{-4}	40±15	145±28

Table II shows that T_3 stimulated lipolysis is effectively blocked by insulin and prostaglandin E_1 but not by the beta adrenergic blocking agent Kö 592 (Stock and Westermann, 1965). Similar results for the effects of insulin and prostaglandin E_1 on theophylline stimulated lipolysis have been described by Rodbell and Jones (1966) and by Steinberg (1967), suggesting that theophylline and T_3 may

have a common mechanism of action. The failure of Kö 592 to block glycerol release due to T_3 indicates that the lipolytic action of T_3 is not mediated via the beta adrenergic receptor.

TABLE II

Effect of Insulin, Prostaglandin E_1 and Kö 592 on T_3 Stimulated Glycerol Release in Isolated Fat Cells

	Glycerol Release (mμmoles/ml/1.5 hr)± SE		
Additions	no T_3	10^{-3}M T_3	10^{-4}M T_3
control	50±2	350±40	260±70
Insulin (Lilly, Iletin) 200μU/ml	27±16	143±25	103±9
Prostaglandin E_1 0.1μg/ml	25±14	77±12	67±12
Kö 592 10^{-4}M	<10	430±60	325±43

The similarity between the data obtained for T_3 and that described by Butcher, et al. (1965) for caffeine and by Hynie, et al. (1966) for theophylline suggested that the mechanism of T_3 stimulated lipolysis also involves an increase in the content of cyclic AMP in adipose tissue. As shown in table III, when rat epididymal fat pads (1 gm tissue/10 mls medium) were incubated for 7 to 12 minutes in the presence of 2.5×10^{-6}M epinephrine (Adrenalin 1:1000, Parke, Davis & Co.) or 10^{-3}M T_3 there were variable increases in the intracellular cyclic AMP content. The combination of these two compounds produced a much greater, though still variable, increase. 3,3'5-triiodo-D-thyronine had a fraction of the activity of the L-isomer in this system.

TABLE III

Effect of Epinephrine and T_3 on Cyclic AMP Levels in Epididymal Fat Pads

	Cyclic AMP (moles x 10^{10}/gm tissue wet wt)		
Additions	Exp. 1	Exp. 2	Exp. 3
control	1.5	1.2	1.6
Epinephrine 2.5×10^{-6}M	3.0	4.2	1.9
T_3 10^{-3}M	2.5	1.5	2.1
3,3'5-triiodo-D-thyronine 10^{-3}M	-	-	1.9
T_3 10^{-3}M plus epinephrine 2.5×10^{-6}M	8.4	15.0	90.0
3,3'5-triiodo-D-thyronine 10^{-3}M plus epinephrine 2.5×10^{-6}M	-	-	2.5

From these results it appears that T_3 raises cyclic AMP in fat tissue by inhibiting the phosphodiesterase which converts cyclic AMP to 5'AMP (Butcher and Sutherland, 1962). Unequivocal data for such a mechanism is presented in table IV where inhibition of both rat adipose tissue and beef heart phosphodiesterase is noted. The inhibition of the beef heart enzyme is apparently competitive. The Ki is of the order of 4×10^{-4}M (Figure 1). The low inhibitory effect of 3,5-diiodo-L-thyronine on the adipose tissue phosphodiesterase probably accounts for its in vitro lipolytic activity (Table 1).

TABLE IV

Inhibition of Phosphodiesterase by T_3 and Related Compounds

	Percent Inhibition of Phosphodiesterase	
Compound (10^{-3}M)	Rat adipose tissue	Beef heart
DL thyroxin	<10	<10
T_3	55	56
3,3',5'-triiodo-D-thyronine	<10	12
3,5-diiodo-L-thyronine	22	<10
L-thyronine	<10	<10

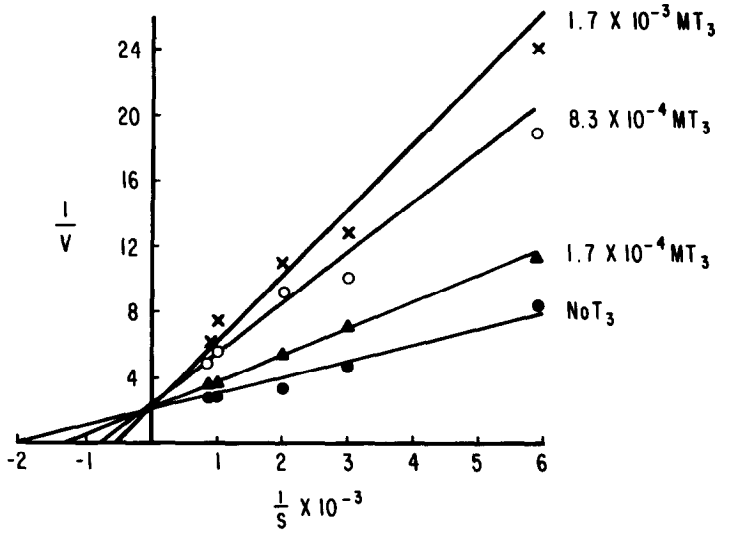

Fig. 1. Inhibition of Beef Heart Cyclic AMP Phosphodiesterase by T_3.

It has been suggested that thyroid hormones might increase the amount of adenyl cyclase, the enzyme forming cyclic AMP, in adipose tissue (Hynie, et al., 1965). The present experiments demonstrate that T_3 exerts its lipolytic action in vitro by preventing the degradation of cyclic AMP. A similar mechanism for the lipolytic action of 3,5-diiodo-L-thyronine in vitro may also exist. Thyroidectomy diminishes the lipolytic response of excised adipose tissue to epinephrine and other adipokinetic hormones; pretreatment of thyroidectomized animals with T_3 restores the lipolytic response (Goodman and Bray, 1966). The present studies raise the possibility that in vivo a function of the thyroid hormones in facilitating the breakdown of triglycerides may be one of preventing the degradation of cyclic AMP. However, T_3 is active in rats at levels corresponding to approximately 10^{-6}M, a concentration well below that required for in vitro activity (Bray and Goodman, 1965). It may be that T_3 is concentrated in adipose tissue. It would be of interest to determine the activity of adipose tissue phosphodiesterase from T_3 treated rats.

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