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INSULIN AND CORTISOL INCREASE THE RESPONSE OF RAT HEPATOCYTES IN PRIMARY CULTURE TO 3,3',5 TRIIODOTHYRONINE

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SUMMARY: Primary cultures of adult rat hepatocytes respond to 3,3',5 triiodothyronine added to the culture media. The specific activities of mitochondrial α-glycerophosphate dehydrogenase and cytosolic malic enzyme are increased. Although the addition of 3,3',5 triiodothyronine causes a 30-70% increase in enzyme activity, the greatest response (150-200% increase) is obtained when both insulin and cortisol are present. Insulin and/or cortisol do not cause any marked increase in either enzyme in the absence of 3,3',5 triiodothyronine.

INTRODUCTION

Current understanding of thyroid hormone actions has largely come from in vivo experiments or work with rat pituitary cell lines (1,2). Primary cultures of rat hepatocytes exhibit numerous characteristic liver functions (3,4). Monolayer cultures of hepatocytes from thyroidectomized rats have been shown to respond to T_3 (5). With this in mind, we have used normal adult rat hepatocyte monolayers to investigate thyroid hormone action. In this paper we describe the response of mitochondrial α-glycerophosphate dehydrogenase (E.C. 1.1.99.5) and cytosolic malic enzyme (E.C. 1.1.1.40) in hepatocyte monolayers to T_3, insulin and

Abbreviations: T_3, 3,3',5 triiodothyronine; PBS^-, phosphate buffered saline, pH 7.4, 8g/l NaCl, 0.2g/l KCl, 0.2g/l KH_2PO_4 1.15g/l Na_2HPO_4; S-Tris-EDTA, 0.25M sucrose, 50 mM Tris-HCl, pH 7.4, 0.1mM EDTA; HEPES, N-(2-hydroxyethyl)-piperazine-N'-2-ethane sulfonic acid; BES, N,N-bis (2-hydroxyethyl)-2-amino ethane sulfonic acid; TES, N-tris (hydroxymethyl) methyl-2-amino ethane sulfonic acid.

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cortisol added to the culture media, and present evidence that insulin and cortisol are required for a maximum response to T_3.

MATERIALS AND METHODS

Adult male Wistar rats with body weights between 200 and 300 g were used in these experiments. The collagenase perfusion method used to isolate hepatocytes was that of Williams (6) with the following modifications. The collagenase perfusion medium consisted of 0.025% collagenase (Type IV, Sigma Chemical Co., St. Louis, Mo.) in L-15 medium (Flow Laboratories Inc., Mississauga, Ont.) containing 5.5mM glucose, 5mM Ca^{++} and antibiotics. Parenchymal cells were isolated from debris and most other cells in 4°C washing medium containing L-15 medium, 5.5mM glucose, antibiotics and 1% BSA (Fraction V, Sigma) (7,8). The hepatocytes were put into culture by inoculating 7.5 x 10^6 viable cells into 15-20ml warmed (37°C) medium in 75 cm^2 Costar tissue culture flasks. The flasks had previously been coated with a film of rat tail collagen (9). The basic culture medium consisted of L-15 medium, 10mM glucose, 1mM succinate, antibiotics and were buffered with 20mM HEPES (Boehringer Mannheim, Dorval, Que) or 10mM HEPES, 10mM TES (Sigma) and 10mM BES (Sigma) at pH 7.4. Any additions to these basic media are described in experimental conditions. T_3, insulin and cortisol were purchased from Sigma Chemical Co. The hepatocytes were allowed to attach to the collagen-coated flasks in media free of T_3. After 2 hours the media containing unattached cells were removed and fresh media with or without T_3 were added. Media were changed every 24 hours thereafter. The hepatocytes were incubated at 37°C in sealed flasks.

Harvesting the hepatocytes for cell fractionation and subsequent enzyme assays was accomplished by treating monolayers washed with PBS^- with 0.05 or 0.1% collagenase in PBS^- for 4 min. Cells were centrifuged at 640 x g at 4°C for 10 min and washed with cold PBS^-. S-Tris-EDTA (1.0 - 1.5ml) was added to the cell pellet which was homogenized using a Polytron (10). The homogenate was centrifuged at 12,100 x g for 10 min and the supernatant termed the Post Mitochondrial Supernatant. The 12,100 x g pellet was suspended in S-Tris-EDTA and the mitochondria were isolated by centrifugation at 5000 x g for 10 min, 10,000 x g for 1.5 min (11), and the pellets were washed once.

α-Glycerophosphate dehydrogenase was assayed in the mitochondrial fraction (12). Malic enzyme was assayed in the Post Mitochondrial Supernatant by a modification of the method of Hsu and Lardy (13). The total assay volume was 1.0ml and contained 3 times the concentrations of L-malate and $NADP^+$. The reaction rate was measured after 8 min.

RESULTS AND DISCUSSION

Hepatic malic enzyme and α-glycerophosphate dehydrogenase show large increases in activity following administration of thyroid hormone to rats (12,14). In order to determine whether rat hepatocytes in culture would respond to T_3 in a similar manner to

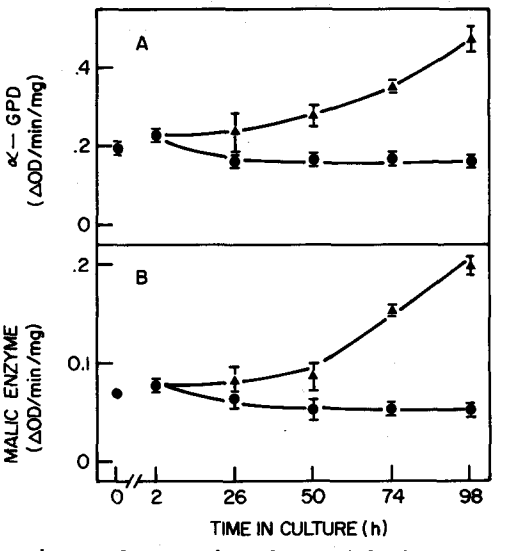

Figure 1. Increase in α-glycerophosphate dehydrogenase and malic enzyme activities in primary cultured hepatocytes by T_3 in the presence of insulin and cortisol. Adult rat liver cells were isolated and cultured as described in methods. All cultures were supplemented with 10^{-5}M cortisol and 10^{-6}M insulin. α-glycerophosphate dehydrogenase (A) and malic enzyme (B) specific activities were measured at the times indicated in cells cultured with 1.54 x 10^{-6}M T_3 (▲ - ▲) and without T_3 (● - ●). Values are means of specific activities determined at two protein concentrations ± range.

the in vivo situation, malic enzyme and α-glycerophosphate dehydrogenase activities were monitored at various times in hepatocytes maintained in media containing 10^{-5}M cortisol, 10^{-6}M insulin and supplemented with or without 1.54 x 10^{-6}M T_3 (1μg/ml). Hepatocytes cultured without T_3 show a slight decrease in α-glycerophosphate dehydrogenase activity over a 4 day period while those with T_3 show a substantial increase (Figure 1 A). Malic enzyme also shows a large increase in activity in cultures maintained in the T_3-supplemented media (Figure 1 B). The time course of the response of α-glycerophosphate dehydrogenase and malic enzyme activities to T_3 in hepatocytes is comparable to that observed in rats receiving thyroid hormones and demonstrates that the concurrent induction of both enzymes occurs in vitro (14). Hepatocyte monolayers rapidly metabolize T_3 (5), and the

TABLE 1. Effects of hormones on α-glycerophosphate dehydrogenase in primary cultured hepatocytes.

Hormones added to culture media	Time in Culture: Expt. A 74 h	Expt. B 70 h	Expt. B 74 h
I. None (Control)	0.114 ± 0.012 (100)†	0.126 ± 0.017 (100)	0.130 ± 0.009 (100)
II. 1.54 x 10^{-6}M T_3	0.197 ± 0.019 (172.5)	0.187 ± 0.023 (149.2)	0.168 ± 0.003 (129.2)
III. 10^{-6}M Insulin 10^{-5}M Cortisol	0.139 ± 0.004 (122.3)	0.120 ± 0.019 (95.5)	0.153 ± 0.022 (117.4)
IV. 10^{-6}M Insulin 10^{-5}M Cortisol 1.54 x 10^{-6}M T_3	0.370 ± 0.042 (342.9)	0.322 ± 0.031 (256.2)	0.390 ± 0.024 (299.8)
V. 10^{-6}M Insulin	0.121 ± 0.031 (105.8)	0.137 ± 0.027 (109.0)	0.141 ± 0.017 (108.1)
VI. 10^{-6}M Insulin 1.54 x 10^{-6}M T_3	0.197 ± 0.010 (173.3)	0.235 ± 0.017 (186.9)	0.272 ± 0.021 (209.2)
VII. 10^{-5}M Cortisol	0.051 ± 0.013 (44.6)	0.083 ± 0.003 (66.4)	0.066 ± 0.008 (50.9)
VIII. 10^{-5}M Cortisol 1.54 x 10^{-6}M T_3	0.170 ± 0.040 (148.8)	0.140 ± 0.019 (111.1)	0.143 ± 0.003 (109.8)

α-glycerophosphate dehydrogenase was assayed as described in methods. Results are expressed as ΔOD/min/mg of the mean of assays at two protein concentrations ± range.

† (% of Control)

concentration of T_3 in the media will vary from the initial 1.54 x 10^{-6}M to a much lower value just before the media are changed. In vivo, maximum induction of malic enzyme and α-glycerophosphate dehydrogenase occurs only when the nuclear T_3 receptors are saturated (15). Thus, the response in cultured hepatocytes to 1.54 x 10^{-6}M T_3 will be dependent on the extent to which the unmetabolized concentration of T_3 in the media between changes saturates the T_3 receptors.

The effect of insulin and cortisol on the T_3-induced increase in α-glycerophosphate dehydrogenase activity (Table 1) and malic

TABLE 2. Effects of hormones on cytosolic malic enzyme in primary cultured hepatocytes.

Hormones added to culture media	Time in Culture Expt. A 74 h	Expt. B 70 h	Expt. B 74 h
I. None (Control)	0.060 ± 0.0 (100)†	0.048 ± 0.005 (100)	0.044 ± 0.005 (100)
II. 1.54 x 10^{-6}M T_3	0.103 ± 0.004 (168.9)	0.070 ± 0.005 (145.8)	0.067 ± 0.011 (152.2)
III. 10^{-6}M Insulin 10^{-5}M Cortisol	0.083 ± 0.004 (136.1)	0.047 ± 0.006 (97.9)	0.049 ± 0.0 (111.4)
IV. 10^{-6}M Insulin 10^{-5}M Cortisol 1.54 x 10^{-6}M T_3	0.345 ± 0.019 (565.6)	0.124 ± 0.0 (258.3)	0.160 ± 0.002 (363.6)
V. 10^{-6}M Insulin	0.094 ± 0.005 (154.1)	0.058 ± 0.008 (120.8)	0.068 ± 0.003 (154.5)
VI. 10^{-6}M Insulin 1.54 x 10^{-6}M T_3	0.173 ± 0.010 (283.6)	0.103 ± 0.005 (214.6)	0.100 ± 0.013 (227.3)
VII. 10^{-5}M Cortisol	0.040 ± 0.037 (65.6)	0.022 * (45.8)	0.021 ± 0.002 (47.7)
VIII. 10^{-5}M Cortisol 1.54 x 10^{-6}M T_3	0.150 ± 0.014 (245.9)	0.049 ± 0.005 (102.1)	0.049 ± 0.001 (111.4)

Malic enzyme was assayed as described in methods. Results are expressed as ΔOD/min/mg of the mean of assays at two protein concentrations ± range.

† (% of Control)

* single assay

enzyme activity (Table 2) was investigated. The maximum response to 1.54 x 10^{-6}M T_3 is approximately a 3-fold increase in α-glycerophosphate dehydrogenase activity and a 2.5 to 5.5-fold increase in malic enzyme activity in hepatocytes maintained in media containing insulin, cortisol and T_3 (IV, Tables 1 and 2) as compared to cultures maintained in media with no hormones (I) or with insulin and cortisol added (III).

Cortisol alone (VII) causes a decrease in malic enzyme and α-glycerophosphate dehydrogenase activities which is reversed by

the addition of T_3 (VIII). It is necessary to include cortisol in the media to obtain maximum stimulation of the enzymes by T_3 since enzyme activities in hepatocytes maintained in media containing insulin and T_3 (VI), although increased, are definitely lower than when cortisol is also present (IV). It appears that cortisol is potentiating the response of hepatocytes to T_3 (in the presence of insulin) in a similar manner to the effects seen for rat pituitary GH_1 cells where the stimulation of growth hormone production by T_3 is greatly increased by cortisol (16,17).

α-Glycerophosphate dehydrogenase and malic enzyme activities in insulin and T_3-supplemented hepatocyte cultures (VI) are increased above those observed in the condition with T_3 alone (II). This implies that insulin also potentiates T_3 action which agrees with the action insulin has been shown to have on malic enzyme induction in chick hepatocytes (18).

In conclusion we report that concurrent increases in α-glycerophosphate dehydrogenase and malic enzyme activities can be demonstrated in normal rat hepatocytes in monolayer culture. Furthermore, the culture system is capable of showing complex interactions between insulin, cortisol and T_3 with respect to enzyme induction.

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