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THE EFFECTS OF CHRONIC ADMINISTRATION OF T_4, GROWTH HORMONE AND EPIDERMAL GROWTH FACTOR ON HEPATIC LIPOGENIC ENZYMES IN HYPOPHYSECTOMISED RATS

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The effect of daily injections of T_4, growth hormone and epidermal growth factor on the activities of the 3 hepatic enzymes malic enzyme (E.C.1.1.1.40), 6 phosphogluconate dehydrogenase (E.C.1.1.1.44) and glucose 6-phosphate dehydrogenase (E.C.1.1.1.49) in hypophysectomised rats was examined. T_4 was shown to increase the activity of malic enzyme in a dose dependent manner from a basal level of 3.55 umol/min/liver to 48.97 umol/min/liver at a dose of 75 ug/Kg/day. A smaller increase in the activity of 6 phosphogluconate dehydrogenase was also observed over the same range of T_4 dosage. Growth hormone in the current study increased the total liver enzyme activity of glucose 6-phosphate dehydrogenase by 84% when given with a replacement dose of 35ug T_4/Kg/day and 6 phosphogluconate dehydrogenase activity by 19% © 1986 Academic Press, Inc.

In the liver the redox states of the $[NAD^+]/[NADH]$ and $[NADP^+]/[NADPH]$ couples have important regulatory functions (1). NADPH acts as a source of reducing equivalents for lipid biosynthesis. In addition, factors changing the rate of lipid biosynthesis are known to affect the the activities of enzymes involved in NADPH generation (2,3,4). Lipogenesis is hormonally regulated, the lipogenic activity of T_3 and insulin being well documented , for review see Geelen and Beynen (5). In addition to these two hormones both GH and EGF have been also been linked with lipid metabolism. In neonatal rats EGF has been reported to stimulate fatty liver formation (6). More specifically in cultured hepatocytes EGF has been shown to stimulate the activity of G6PDH, a key enzyme involved in lipogenesis while supressing insulin stimulated lipid biosynthesis (7). The GH molecule has been reported to possess lipolytic activity (8). In contrast with this study is the report that GH administration to hypophysectomised rats causes an increase in the $[NADP^+]/[NADPH]$ ratio in the livers of these animals (9). As malic enzyme, 6PGDH and G6PDH are the main NADPH generating enzymes, responsible for the supply of reducing equivalents needed

Abbreviations: EGF, epidermal growth factor. GH, growth hormone. G6PDH, glucose 6-phosphate dehydrogenase. 6PGDH, 6 phosphogluconate dehyrogenase.

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during lipogenesis we have undertaken a controlled comparative study on the effects T_4, EGF and GH on the activities of these 3 enzymes.

MATERIALS AND METHODS

Materials: L-Thyroxine was purchased from Calbiochem-Behring corporation La Jolla, CA. Corticosterone-21-acetate and deoxycorticosterone acetate were purchased from Sigma Chemical Co. St. Louis, MO. EGF was from Collaborative Research Inc. Lexington, MA. Bovine somatotropin was purchased from Miles Scientific Co., Naperville, IL.
Animals and treatments: Female Sprague Dawley (Zivic Miller strain) were used in all experiments, (Zivic Miller Labs, Allison Park, PA.). The hypophysectomised animals were 24 days old at time of operation. The pituitary was surgically removed by aspiration the operation being performed at Zivic Miller Lab. and animals housed there during treatment period. Beginning 3 days after operation and continuing for 10 days, subcutaneous injections of replacement hormones were given to hypophysectomised animals at the following dosage, corticosterone-21-acetate (1mg/Kg/day), deoxycorticosterone (20ug/Kg/day) and T_4, GH or EGF at the appropriate experimental dosage. All injections were given in saline and the dose adjusted daily following weighing of the animals. The hypophysectomised animals received a Zeigler low iodine diet *ad lib* (Zeigler Co., Gardner, PA.) and a replacement salt supplement in their drinking water (10). The intact control animals received saline injections and were maintained on the same low iodine diet for 3 days prior to sacrifice. During this period their drinking water was supplemented with iodine to a normal dietary level.
Enzyme assays: The frozen livers were ground under liquid N_2 to a powder. Aliquots of the liver powder were removed and homogenised in 1mM EDTA. Appropriate dilutions of the homogenate were assayed directly. Malic enzyme was assayed by the method of Hsu and Lardy (11) and 6PGDH and G6PDH assayed by the method of Rudack *et al* (12) at 37°C.

RESULTS

The effect of T_4 on the activities of malic enzyme, 6PGDH and G6PDH: As expected T_4 increased the activity of malic enzyme in a dose dependent manner over the range of replacement doses tested (Fig. 1). The basal malic enzyme activity in animals receiving no T_4 supplement was 3.55 umol/min/liver (1.26 umol/min/g liver). This increased to a value

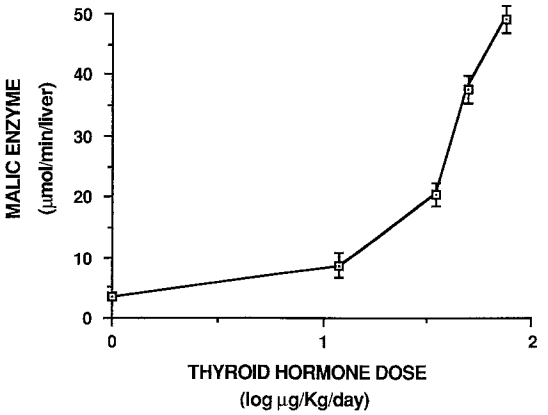

Figure 1. The effect of T_4 administration on total hepatic malic enzyme activity in hypophysectomised rats. All data points are represented ± standard error of the mean. For n see table 1.

TABLE 1

The Effect of Thyroid Hormone on the Activity of 6 Phosphogluconate Dehydrogenase and Glucose 6-Phosphate Dehydrogenase in Rat Liver

	n	Liver wt. (g)	6PGDH		G6PDH	
			umol/min			
			/g liver	/total liver	/g liver	/total liver
Intact control[a]	4	3.33 ± 0.14	11.68 ± 1.39	38.43 ± 4.00	8.41 ± 0.96	27.66 ± 2.47
T_4 ug/Kg/day						
0	5	2.80 ± 0.11	7.72 ± 0.74	21.41 ± 1.95	3.93 ± 0.73	10.97 ± 1.95
12	5	2.70 ± 0.08	10.06 ± 1.23	27.36 ± 3.72	3.12 ± 0.30	8.49 ± 0.95
35	5	2.50 ± 0.08	11.22 ± 0.43	27.87 ± 0.68	3.15 ± 0.44	7.87 ± 1.08
50	5	3.14 ± 0.09	11.99 ± 0.45	37.59 ± 1.44	4.06 ± 0.16	12.84 ± 0.73
75	5	2.78 ± 0.07	14.20 ± 0.61***	39.42 ± 1.59***	3.01 ± 0.50	8.25 ± 1.19

Following removal of the pituitary gland, the animals received a standard replacement dose of corticosterone 1-acetate (1 mg/Kg/day), and deoxycorticosterone (20 ug/Kg/day) for 10 days. Thyroid hormone T_4 was given at the dose specified above during this period. At the end of the treatment period, the animals were sacrificed and their livers quick frozen prior to extraction and assay of the enzyme activities (7,8). All values reported ± S.E.M.

[a]Non hypophysectomized animals.

*** $p \leq 0.001$.

of 48.97 umol/min/liver (17.61 umol/min/g liver) in animals receiving a T_4 dose of 75ug/Kg/day for 10 days. The mean malic enzyme activity in intact animals was 20.52 umol/min/liver and this was approximated in experimental animals receiving a T_4 replacement of 35 - 50 ug/Kg/day over a 10 day period. Accordingly these 2 doses were chosen as replacement levels for other experiments. The activity of 6PGDH was significantly increased in the hypophysectomised animals receiving a high dose of T_4 for 10 days (Table 1). At 0 ug/Kg/day T_4 the activity of this enzyme was 21.41 umol/min/liver (7.72 umol/min/g liver) and this increased to 39.42 umol/min/liver (14.2 umol/min/g liver) at a dose of 75 umol/Kg/day. G6PDH did not increase over the range of T_4 tested (Table 1).

The Effect of GH and EGF on the activities of malic enzyme, 6PGDH and G6PDH: Based on the results obtained in the T_4 dose response study, GH and EGF were tested in animals receiving either a 35 or 50 ug/Kg/day T_4 replacement dose. GH (Miles, bovine pituitary) was injected daily at a dose of 3mg/Kg/day. There was no significant change in the activity of malic enzyme measured at either T_4 dose following administration of GH (Table 2). However the total liver activity of G6PDH increased by 84% in the animals receiving a

TABLE 2

The Effect of Growth Hormone and Epidermal Growth Factor on the Activities of Malic Enzyme, 6 Phosphogluconate Dehydrogenase and Glucose 6-Phosphate Dehydrogenase in Rat Liver

Treatment	n	Liver wt (g)	Malic enzyme umol/min		6PGDH umol/min		G6PDH umol/min	
			/g liver	/total liver	/g liver	/total liver	/g liver	/total liver
Control 35 ug T_4	5	2.50 ± 0.08	8.29 ± 0.77	20.31 ± 1.86	11.22 ± 0.43	27.87 ± 0.68	3.15 ± 0.44	7.87 ± 1.08
GH[a] + 35 ug T_4	5	3.16 ± 0.14	8.62 ± 0.95	27.39 ± 3.58	10.52 ± 1.41	33.44 ± 4.27	4.60 ± 0.47	14.49 ± 1.88*
Control 50 ug T_4	5	3.14 ± 0.09	11.98 ± 0.76	37.54 ± 2.33	11.99 ± 0.45	37.59 ± 1.44	4.06 ± 0.16	12.84 ± 0.73
GH + 50 ug T_4	5	3.34 ± 0.09	13.70 ± 1.42	45.93 ± 5.02	13.35 ± 0.41	44.67 ± 2.28*	4.30 ± 0.40	14.49 ± 1.68
EGF Control 50 ug T_4	7	2.83 ± 0.09	9.01 ± 1.32	24.95 ± 3.46	9.81 ± 0.74	27.52 ± 1.71	4.10 ± 0.19	11.64 ± 0.76
EGF[b] + 50 ug T_4	7	3.14 ± 0.08	5.53 ± 0.67*	17.52 ± 2.37	9.81 ± 0.92	30.69 ± 2.76	2.49 ± 0.26***	7.78 ± 0.71**

All animals following removal of the pituitary received a standard replacement dose of corticosterone 1-acetate (1 mg/Kg/day) and deoxycorticosterone (20 ug/Kg/day) for 10 days following surgery. In addition, animals received a daily thyroid hormone supplement at the dosage indicated above. Experimental treatment groups were injected daily with either (a) growth hormone 3 mg/Kg/day or (b) EGF 40 ug/Kg/day. All values are reported ± S.E.M.

* $p \leq 0.05$.

** $p \leq 0.01$.

*** $p \leq 0.001$.

35ug/Kg/day replacement dose of T_4 ($p \leq 0.02$) while 6PGDH activity was slightly elevated by 19% in the group receiving a higher T_4 replacement dose of 50ug/Kg/day ($p \leq 0.05$) (Table 2). There was no significant increase in either of these enzyme activities following GH administration when the enzyme activity was expressed on a units/ g liver basis. In contrast EGF at a dose of 40ug/Kg/day caused a 50% decrease in G6PDH activity. The enzyme activity decreased from a control value of 11.64 umol/min/liver (4.1 umol/min/g liver) to 7.78 umol/min/liver (2.49 umol/min/g liver) in the treated animals ($p \leq 0.01$). A slight decrease in the activity of malic enzyme was also noted when expressed on a units/g liver basis. No change was noted in 6 phosphogluconate dehydrogenase after treatment for 10 days with EGF.

DISCUSSION

In the present study we have demonstrated in hypophysectomised rats a dose dependent response of malic enzyme to T_4. An induction of malic enzyme in response to thyroid hormone has previously been reported (13,14) and been shown by Mariash *et al* (15) to be mediated by increased synthesis of the corresponding mRNA. We have also shown a lesser effect of this hormone on the activity of 6PGDH. This enzyme activity being significantly increased over the range of T_4 tested in the animals. However T_4 did not appear to affect the total liver activity of G6PDH. The differential effect of T_3 on these 3 enzyme activities has been noted by Mariash *et al* (15). Miksicek and Towle (16) also reported a lesser effect of T_3 on the induction of G6PDH activity as compared to the increases in the activities of malic enzyme and 6PGDH.

A previous study on hypophysectomised rats treated with GH reported an increase in the ratio of free cytoplasmic $[NADP^+]/[NADPH]$ (9). This finding is consistent with increased utilization of NADPH for such anabolic processes as lipid and nucleotide biosynthesis. In contrast other studies have demonstrated a lipolytic activity associated with GH (8,17-19). If lipolysis is occuring a decrease in the ratio of $[NADP^+]/[NADPH]$ might be expected. In the present investigation we have shown under our experimental conditions no effects of GH on the total liver activity of malic enzyme and an apparently T_4 dependent effect of this hormone on the activities of G6PDH and 6PGDH *in vivo*. Previously Glock and McClean (20) showed no effect of GH on the total liver activities of G6PDH and 6PGDH *in vivo*, as the current effects appear to depend on the hormonal status of the animal this discrepancy is perhaps explainable. However the results obtained in the present study contrast with reported changes in cultured hepatocytes where GH has been shown to effect the activities of both malic enzyme and G6PDH (21).

The effect of EGF was found to be opposite to that of GH . A decrease in G6PDH activity occurring. As G6PDH occupies a key metabolic location, important for regulating both lipid and nucleic acid biosynthesis it is an appropriate site of action for EGF. A slight decrease in the units of malic enzyme activity /g liver was also observed although the total liver activity of this enzyme did not change. EGF is known to stimulate DNA biosynthesis in hepatocytes (22) and in addition has been shown to stimulate G6PDH activity (7). The same authors also report a suppression of insulin and T_3 stimulation of malic enzyme caused by EGF suggesting an anti-lipolytic action. In a previous study Heimberg *et al* (6)

EGF has been shown to promote lipid deposition in the livers of neonatal rats. It has been demonstrated in a further investigation from this laboratory (23) that one of the earliest effects of this agent is to elevate the level of G6P *in vivo* although in the previous study no change in G6PDH activity was found at 5 min after EGF administration. In the present investigation the physiological significance of the EGF induced decrease in G6PDH remains to be elucidated. This study however, demonstrates that despite the common function of T_4, GH and EGF in the maintenance of growth and development each factor appears to affect the 3 hepatic enzymes studied in a different and specific manner.

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