EFFECTS OF ADRENALECTOMY AND CORTISOL TREATMENT ON DIHYDROXYACETONE PHOSPHATE ACYLTRANSFERASE ACTIVITY IN RAT LIVER

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Received 31 March 1981

1. Introduction

Currently it is thought that the biosynthesis of glycerolipids is initiated by the acylation of glycerol phosphate or dihydroxyacetone phosphate. The relative contributions of these two metabolic routes are not fully established (reviewed in [1]). There is believed to be a peroxisomal dihydroxyacetone phosphate acyltransferase (DHAPAT) activity in liver [2,3] although on subcellular fractionation DHAPAT activity is also found in mitochondria and microsomes. Glycerol phosphate acyltransferase (GPAT) activity is found in both mitochondrial and microsomal fractions (reviewed in [4]). We have shown that mitochondrial GPAT activity is decreased by adrenalectomy in both fed and fasted animals [5]. Microsomal GPAT activity was decreased by adrenalectomy in fasted animals only [5]. These results show the effects of adrenalectomy and cortisol injection on hepatic DHAPAT activity.

2. Methods

Sources of enzymes and chemicals are described in [5]. In addition cortisol (hydrocortisone 21-acetate) was obtained from Sigma (London) Chemical Co. (Kingston-upon-Thames, Surrey).

Adrenalectomised rats and their sham-operated controls were purchased from two sources, Olac Ltd. (Bicester) and Charles River (Margate, Kent) supplied the animals for exp. 1 and exp. 2, respectively. The operations were performed on 130 g male Sprague-Dawley rats. Animals were used 8–12 days after the operation. They had constant access to food and water, adrenalectomised animals being given 0.9% (w/v) NaCl in tap water to drink. Fasted animals had food removed 24 h before sacrifice.

In exp. 1 animals were injected subcutaneously twice daily (at about 10:00 and 17:00 h) for 2 days prior to sacrifice. These injections consisted of cortisol dissolved in 0.9% (w/v) NaCl at 50 mg/kg body wt or an equivalent volume of 0.9% (w/v) NaCl. Rats were killed by cervical dislocation. In exp. 1 samples of liver were freeze-clamped between aluminium plates precooled in liquid N_2 [6] and stored under liquid N_2. The remaining liver was removed, weighed and homogenised in a known volume of 0.25 M sucrose, 10 mM Tris–HCl, 1 mM EDTA (pH 7.4) (medium A) as in [5]. An aliquot of this homogenate was stored frozen at −20°C for protein determination. Tissue extracts of freeze-clamped liver were prepared as in [5] and assayed for DHAPAT activity [5].

In exp. 2 livers were flushed through with 50 ml ice-cold 0.25 M sucrose, 10 mM Tris-HCl, 1 mM EGTA (pH 7.4) (medium B) before removal from the animal. The livers were minced with scissors in ice-cold medium B and washed 3 times with ~50 ml medium B before homogenisation in 50 ml medium B supplemented with 1% (w/v) fatty acid-poor albumin as in [5]. Samples of homogenate were stored frozen at −20°C for determination of DHAPAT [5] and uricase [7] activities. The homogenate was centrifuged at 7250 × *g* for 10 min and the pellet discarded. The supernatant was recentrifuged at 23 000 × *g* for 10 min and the peroxisomal/lysosomal pellet washed twice with 30 ml medium A by resuspension and recentrifugation. The resultant pellet was resuspended in medium A at 4–8 mg protein/ml and stored frozen in aliquots at −20°C. DHAPAT and uricase activities were subsequently determined. Protein was assayed [8] using bovine serum albumin as standard.

3. Results and discussion

Table 1 shows that DHAPAT activity/total liver is significantly decreased by 44% in adrenalectomised animals and that injection of cortisol restores the activ-

Table 1
Effect of adrenalectomy and cortisol injection of DHAPAT activity in Ultra-Turrax homogenates of freeze-stopped livers (means ± SE)

Exp. 1	Sham-operated (saline-injected)	Adrenalectomised (saline-injected)	Adrenalectomised (cortisol-injected)
No. of animals	5	6	6
Body weight (g)	206 ± 5	179 ± 8[a]	185 ± 7
Liver weight (g)	9.8 ± 0.8	7.1 ± 0.3[b]	9.2 ± 0.6[e]
Total liver protein (g)	1.76 ± 0.11	1.20 ± 0.03[d]	1.52 ± 0.10[e]
DHAPAT activity (nmol/min)			
Per total liver	248 ± 31	140 ± 4[c]	306 ± 12[f]
Per mg liver protein	0.14 ± 0.01	0.12 ± 0.0	0.20 ± 0.01[f]

Effects of adrenalectomy: [a–d] $p < 0.05$, 0.02, 0.01 and 0.001, respectively
Effects of cortisol: [e,f] $p < 0.02$ and 0.001, respectively

ity to levels seen in sham-operated animals. When these results are expressed/mg liver protein the effect of adrenalectomy is no longer apparent. This results from a decrease in total liver protein in adrenalectomy. The effect of cortisol, however, is still apparent. These alterations in DHAPAT activity may be related to the decrease in the synthesis and export of hepatic triacylglycerol after adrenalectomy [9,10] and the increase after glucocorticoid therapy [9,11]. It is of interest that hepatic GPAT activity has also been shown to respond to adrenalectomy [5].

N-Ethylmaleimide (NEM) stimulates DHAPAT activity in peroxisomal and mitochondrial [5,12] subcellular fractions but decreases that in microsomal fractions [5,13]. Assays performed in the presence of NEM should therefore estimate an activity in which the contribution from a microsomal enzyme is small. In exp. 2 (table 2) DHAPAT activity was assayed in the absence and presence of NEM to test whether the effect of adrenalectomy could be enhanced (a) by NEM treatment and (b) in a 23 000 × *g* peroxisomal/lysosomal fraction. In addition DHAPAT activity was determined after 24 h fasting.

When DHAPAT activity was assayed in this 23 000 × *g* fraction the stimulation by NEM was 60 ± 4% (table 2). Using homogenates a 10 ± 3% stimulation resulted presumably indicating the presence of an appreciable NEM-inhibited activity which may be microsomal in origin [5,13]. The effect of adrenalectomy seen in table 1 was also apparent when liver samples were disrupted without freezing using a Potter-Elvehjem homogeniser (table 2). The decrease in DHAPAT activity was, however, smaller being 28% and 23% for assays performed in the absence and presence of NEM, respectively. After 24 h fasting similar assays gave decreases in DHAPAT activity of 34% and 24%. When a 23 000 × *g* fraction was prepared from these livers the effects of adrenalectomy and fasting persisted but were not enhanced in this fraction either in the absence or presence of NEM.

These results indicate that the effects of adrenalectomy and fasting cannot be confined to the peroxisomal fraction or to activities that are NEM-inhibited such as the microsomes. The decrease in DHAPAT activity after adrenalectomy seen with freeze-stopped tissue (table 1) is greater than that seen when livers are homogenised directly (table 2). This could be the result of different experiments or animals or indicate the existence of some non-persistant effect. Previously work has shown that fasting for 48 h causes a 24% decrease in DHAPAT activity measured in Ultra-Turrax homogenates from freeze-stopped tissue [5] which is comparable to that seen here with 24 h fasting.

Uricase activity is also affected by adrenalectomy and fasting (table 2). The decrease is 43% and 50% for adrenalectomy in fed and fasted animals, and 29% and 38% for fasting in sham-operated and adrenalectomised animals, respectively. This adaptivity by uricase activity is larger than that seen for DHAPAT activity resulting in a decrease in the ratio of DHAPAT to uricase activity in the 23 000 × *g* fraction. Hepatic uricase activity has been shown to be unchanged by diabetes (E. J. B., E. D. S., unpublished).

These results demonstrate clearly that hepatic DHAPAT activity is decreased after adrenalectomy. This effect is reversed by cortisol therapy. The changes are in the same direction as those in triacylglycerol synthesis and export [9–11]. Other enzymes of tri-

Table 2
Effect of adrenalectomy on DHAPAT activity in fed and fasted animals (means ± SE)

Exp. 2	FED		FASTED	
	Sham-operated	Adrenalect-omised	Sham-operated	Adrenalect-omised
No. animals	5	5	4	5
Body weight (g)	194 ± 3	177 ± 5[b]	183 ± 6	165 ± 8
Homogenate activities:				
DHAPAT (nmol . min^{-1} . total liver^{-1})				
without NEM	170.0 ± 10.1	122.1 ± 3.5[b]	147.8 ± 8.6	97.8 ± 10.8[b]
with NEM	191.9 ± 9.8	139.3 ± 5.3[b]	152.3 ± 9.5	115.0 ± 14.4
Uricase (μmol . min^{-1} . total liver^{-1})	6.96 ± 0.75	4.00 ± 0.36[b]	4.92 ± 0.55	2.47 ± 0.46[ac]
23 000 × g fraction activities:				
[f]DHAPAT (nmol . min^{-1} . total liver^{-1})				
without NEM	74.0 ± 13.1	54.4 ± 8.3	56.1 ± 9.2	42.2 ± 4.4
with NEM	111.8 ± 18.8	83.9 ± 12.5	89.2 ± 16.7	71.3 ± 6.4
DHAPAT (nmol . min^{-1} . mg protein^{-1})				
without NEM	0.585 ± 0.056	0.544 ± 0.043	0.566 ± 0.067	0.700 ± 0.024[d]
with NEM	0.884 ± 0.072	0.838 ± 0.061	0.902 ± 0.131	1.193 ± 0.057[e]
Uricase (nmol . min^{-1} . mg protein^{-1})	57.8 ± 7.5	42.0 ± 4.8	50.7 ± 6.5	41.0 ± 6.4
Ratio uricase/DHAPAT activities:				
without NEM	104.6 ± 18.1	80.6 ± 13.0	90.0 ± 6.8	57.7 ± 7.4[a]
with NEM	68.5 ± 11.5	52.2 ± 8.6	57.4 ± 5.1	33.8 ± 4.1[b]

Effects of adrenalectomy: [a,b] $P < 0.02$ and 0.01, respectively
Effects of fasting; [c–e] $P < 0.05$, 0.02 and 0.01, respectively

[f] DHAPAT activity was calculated from a knowledge of the uricase activity of the fraction together with the uricase activity of the total liver

DHAPAT activity assays were performed in the absence and presence of 10 mM NEM

acylglycerol synthesis namely GPAT [5] and phosphatidate phosphohydrolase [14] also decrease in activity after adrenalectomy. DHAPAT is the first enzyme in the synthesis of lipids containing both ester and ether bonds and changes in its activity may be important in the regulation of either of these pathways.

Acknowledgement

We thank the Medical Research Council for financial support.

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