ACUTE EFFECTS OF INSULIN ON GLYCEROL PHOSPHATE ACYL TRANSFERASE ACTIVITY, KETOGENESIS AND SERUM FREE FATTY ACID CONCENTRATION IN PERFUSED RAT LIVER

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1. Introduction

In the perfused rat liver insulin inhibits the oxidation of serum free fatty acids [1] and endogenous fatty acids [2], increases the secretion of very low density lipoprotein triacylglycerol [1–3] and also opposes the antilipogenic effect of long chain free fatty acids [3]. In isolated adipocytes insulin increases the synthesis of triacylglycerol. Although some of this insulin effect in adipose tissue may be attributed to enhancement of glucose transport and hence provision of triose phosphates for esterification, there is evidence for more direct insulin action upon glyceride synthesis [4]. In vitro adipocyte glycerol phosphate acyltransferase (GPAT) and long-chain fatty acyl CoA synthetase activities have been shown to increase as a result of insulin treatment [5–7]. It was therefore of interest to examine the acute effects of insulin on activities of these enzymes in the perfused rat liver.

2. Materials and methods

Livers from fed male Wistar albino rats (340–360 g) were perfused in situ for an experimental period of 30 min by a modification of the method [8]. The perfusate was 80 ml defibrinated whole rat blood dialysed against a modified Krebs bicarbonate buffer [9] containing glucose (12 mM), mixed amino acids (500 mg/litre) and 1.27 mM Ca^{2+}. The rate of perfusion was 12 ml blood/min (i.e., approx. 1 ml/g liver/min) and the PO_2 of blood entering the liver was maintained at 12–13 *K* Pa. Bovine insulin was infused in 0.15 M NaCl to maintain constant concentrations of 80–100 ng/ml blood. Control livers were infused with 0.15 M NaCl in place of the insulin solution. At the end of perfusion portions of the liver were taken by a freeze-stop technique [10] and stored under liquid N_2. The same liver lobe was always sampled. Portions of frozen liver (approx. 0.5 g) were powdered in a mortar under liquid N_2 and homogenised with an Ultra-Turrax tissue disintegrator (3 × 10 s bursts over 1 min) in 5 ml ice-cold 0.25 M sucrose, 10 mM Tris–chloride 1 mM EDTA, 1 mM dithiothreitol, pH 7.4. GPAT and fatty acyl CoA synthetase assays were performed immediately on portions of whole homogenates. Methods for assay of Acyl CoA, L-glycerol 3 phosphate-*o*-acyltransferase (EC 2.3.1.15) (in the presence of 6 mg/ml fatty acid-poor albumin) and glutamate dehydrogenase (EC 1.4.1.2) are described [11]. Long chain fatty acyl CoA synthetase (EC 6.2.1.3) was assayed at 30°C by following the incorporation of [^{3}H]coenzyme A into palmitoyl CoA as described [12]. All assays were linear with respect to both time and protein concentration. Protein was measured in portions of whole homogenates by the method [13].

Concentrations of blood ketone bodies (D-3-hydroxybutyrate and acetoacetate) were determined enzymatically [14,15] and serum free fatty acids by the method [16].

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Table 1
Effect of insulin on activities of glycerol phosphate acyltransferase and fatty acyl CoA synthetase in perfused rat liver

Glycerol phosphate acyltransferase activity (nmol/min/mg protein)					Fatty acyl CoA synthetase activity (nmol/min/mg protein)	
Glycerol phosphate conc. (mM)	Insulin (80–100 ng/ml blood)	Total activity	NEM-insensitive activity	NEM-sensitive activity	Insulin (80–100 ng/ml blood)	Total activity
0.5	–	0.596 ± 0.028	0.347 ± 0.027	0.250 ± 0.003	–	7.20 ± 1.66
	+	0.739 ± 0.028[c]	0.466 ± 0.030[a]	0.273 ± 0.009	+	8.45 ± 1.77
5.0	–	1.302 ± 0.082	0.962 ± 0.070	0.340 ± 0.012		
	+	1.599 ± 0.044[b]	1.220 ± 0.060[a]	0.379 ± 0.034		

[a] $P < 0.05$
[b] $P < 0.02$
[c] $P < 0.01$

The results are means ± SEM of 4 experiments without insulin and 5 with insulin

Statistical analysis of data was by Student's *t*-test. Variations are indicated as the SEM.

3. Results and discussion

Table 1 shows that total GPAT activity/mg liver protein was significantly elevated in livers perfused for 30 min with insulin concentrations which were not significantly different from those found in blood of rats after carbohydrate loading [17]. The increase due to insulin was 23% when 5 mM [^{14}C]glycerol phosphate was used in the assay and 24% with 0.5 mM [^{14}C]glycerol phosphate. The latter concentration is similar to the total issue concentration of glycerol phosphate found in rat liver (Topping, D. L., unpublished observations). The activity/mg protein observed with 5 mM glycerol phosphate was similar to [18] under comparable assay conditions. Liver protein was 151 ± 8 and 147 ± 3 mg/g wet wt for livers perfused without or with insulin, respectively.

Microsomal GPAT activity is sensitive to thiol reagents [19–21], whereas mitochondrial activity is reported to be insensitive to, or possibly slightly stimulated by these agents [20,21]. These effects were essentially confirmed when isolated rat liver mitochondria and microsomes were frozen in liquid N_2, homogenised and assayed for GPAT activity with or without 10 mM *N*-ethylmaleinide (NEM). NEM 10 mM, decreased mitochondrial GPAT activity by 10% or less when assayed with 0.5 mM [^{14}C]glycerol phosphate, but decreased microsomal activity 85–90% under the same conditions (Bates, E. J., unpublished observations). GPAT activity observed in liver homogenates that was insensitive to 10 mM NEM was therefore considered to represent an enrichment of mitochondrial activity over that seen in the absence of the thiol reagent. Conversely, the NEM-sensitive activity calculated by difference was considered to represent a relative enrichment of microsomal activity. At 0.5 mM glycerol phosphate the increase in NEM-insensitive GPAT activity with insulin was 34% whereas the NEM-sensitive activity was only increased by 9%. This suggests that most of the insulin response resides in the mitochondrial GPAT under the present conditions. It is noteworthy that liver mitochondrial GPAT is selectively decreased in mild diabetes [11].

Glutamate dehydrogenase was also assayed as a standard mitochondrial enzyme activity unlikely to be affected by insulin – which was in fact the case. Livers perfused without added insulin contained 0.92 ± 0.04 units/mg protein (mean ± SEM) and those perfused with insulin contained 0.91 ± 0.06 units/mg protein. Total tissue activity of long chain fatty acyl CoA synthetase however was not significantly changed by insulin. This may suggest that this enzyme is not involved in the regulation of hepatic fatty acid metabolism by insulin, which is supported by the observation that insulin does not change the fractional extraction of infused serum free fatty acids by the perfused liver but does alter the balance between the fatty acid oxidation and esterification pathways [1].

In addition to increasing GPAT activity insulin also significantly lowered the steady state concentration of serum free fatty acids in the perfusate and appreciably decreased net ketone body production (table 2). It should be noted that there was no infusion of exogenous free fatty acid during these experiments. If the serum free fatty acids are in equilibrium with intracellular free fatty acids under these conditions, the change in serum free fatty acid concentration presumably represents a change in the balance between

Table 2
Effect of insulin on serum-free fatty acid concentration and ketogenesis in perfused rat liver

Insulin (80–100 ng/ml blood)	Free fatty acid conc. (μmol/mol serum)	Net ketone production bodies (μmol/liver/h)
–	0.175 ± 0.006 (4)	23.2 ± 3.9 (4)
+	0.156 ± 0.005[a] (5)	11.3 ± 3.7[b] (4)

[a] $P < 0.05$
[b] $0.05 < P < 0.1$

fatty acid utilisation and release from intracellular triacylglycerol stores. The observed effect of insulin on free fatty acid concentration and ketone body production may be indicative, therefore, of a change in the balance betweeen fatty acid oxidation and esterification resulting from the increase in mitochondrial GPAT activity. Alternatively, or in addition, the observed increase in GPAT with insulin might be secondary to a primary action of insulin upon lipolysis [1]. Further experiments are necessary to elucidate the nature of this insulin action.

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