Effects of corticosterone and insulin on enzymes of triacylglycerol synthesis in isolated rat hepatocytes

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1. INTRODUCTION

The rate of hepatic triacylglycerol synthesis can be influenced by both insulin and glucocorticoids. Insulin has been suggested to stimulate triacylglycerol synthesis in the liver by increasing the supply of substrates for esterification, redirecting palmitoyl-CoA esters from oxidation to esterification [1], and by increasing glycerol 3-phosphate acyltransferase (EC 2.3.1.15) activity [2–4]. Stimulation of hepatic triacylglycerol synthesis by glucocorticoids has been mainly attributed to increases in phosphatidate phosphohydrolase (EC 3.1.3.4) activity [5], which has been demonstrated with isolated perfused livers [6] and hepatocytes [7]. The corticosterone-induced stimulation of phosphatidate phosphohydrolase activity has been shown to be inhibited by insulin [8]. In addition to the effects on phosphatidate phosphohydrolase, there is indirect evidence from experiments in vivo that the mitochondrial and microsomal glycerol phosphate acyltransferase activities may be increased by glucocorticoids [3,9,10]. Thus the aims of the work presented here were 3-fold:

(i) To determine whether the capacity to synthesize neutral lipids by hepatocyte homogenates mirrored changes in phosphatidate phosphohydrolase activity;
(ii) To measure the total capacity of esterification in vitro, and to see if this could be altered by insulin or corticosterone.
(iii) To determine if any other enzyme activities involved in esterification, besides the phosphohydrolase, could be altered by the hormonal challenges.

In summary, the corticosterone-induced stimulation of phosphatidate phosphohydrolase activity was accompanied by a 2.9-fold increase in the capacity to synthesize neutral lipids from glycerol phosphate and palmitate. There was a 1.6-fold increase in the capacity to esterify glycerol 3-phosphate into all lipid classes. Insulin antagonized all of these corticosterone-induced stimulations. No individual enzyme involved in esterification, besides the phosphohydrolase, was significantly altered in activity by the hormone treatments.

2. MATERIALS AND METHODS

2.1. *Materials*

Most of the materials have been described in [7–9,11] except for bis-(*p*-nitrophenyl)phosphate which was obtained from Sigma Chemicals. The sample of partially purified phosphatidate phosphohydrolase was a gift from R. Hopewell and S. Butterwith. It was purified 100-fold from the soluble fraction of the livers of ethanol-treated rats [12] and had spec. act. 507 nmol phosphatidate hydrolysed . min^{-1} . mg $protein^{-1}$.

2.2. *Methods*

The methods for preparing and incubating hepatocytes, the determination of phosphatidate phosphohydrolase activity, the determination of lactate dehydrogenase to account for cell breakage

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and the characterization of the cells have been described [7,8].

Diacylglycerol acyltransferase (EC 2.3.1.20) activity was measured by a method based upon that in [13,14]. Each assay contained in 0.2 ml final vol. 175 mM Tris, adjusted to pH 8.0 with HCl, 30 μM [9,10-^{3}H]palmitoyl-CoA (spec. act. 100 Ci/mol), 0.15 mM 1,2-*sn*-diacylglycerol (added in 10 μl ethanol), 8 mM $MgCl_2$, 1 mg fatty-acid-poor bovine serum albumin/ml and 4 mM bis-(*p*-nitrophenyl)-phosphate. The last constituent was included to inhibit ethanol acyltransferase activity [13]. Assays were started with 40–120 μl hepatocyte homogenate and terminated after 3 min at 23°C with 1.5 ml propan-2-ol/heptane/H_2O (80:20:2, by vol.). The extraction and determination of products has been described [13]. Employing TLC [11] all the radioactivity was located in the band corresponding to triacylglycerol. Ethyl-[9,10-^{3}H]palmitate could not be detected.

The measurements of glycerol 3-phosphate acyltransferase activity [9] and acyl-CoA synthetase (EC 6.2.1.3) activity [15] have been described, except 0.15 mM CoA was used in assaying acyl-CoA synthetase activity.

The total esterification of glycerol 3-phosphate was measured at 37°C in 0.25 ml final vol. containing 25 mM Tris, adjusted to pH 7.4 with HCl, 5 mM dithiothreitol, 2.5 mM $MgCl_2$, 2.5 mM ATP, adjusted to pH 7.4 with $KHCO_3$, 0.15 mM CoA, 0.8 mM K-palmitate (in a 20% molar excess of KOH), 8 mM [1,3-^{3}H]glycerol 3-phosphate (spec. act. 4 Ci/mol), 6 mg fatty-acid-poor bovine serum albumin/ml and 90–180 μl hepatocyte homogenate. Assays were terminated after 20 min by the addition of 1.88 ml chloroform/methanol (1:2, v/v). Lipids were extracted [16] and analysed by either aluminium oxide column chromatography [17] or by TLC [18].

3. RESULTS AND DISCUSSION

The rates of esterification and the enzyme activities involved in triacylglycerol synthesis in homogenates of hepatocytes incubated for 6 h at 37°C [7,8] were defined as control values and are given in table 1. Incubating hepatocytes for 6 h with 10^{-5} M corticosterone increased phosphatidate phosphohydrolase activity by 1.8-fold, and this effect was antagonized by 20 mU/ml insulin (table 1). Insulin (20 mU/ml) alone had no significant effect upon phosphohydrolase activity. These results were expected from [7,8] and are given for comparative purposes.

Corticosterone (10^{-5} M) stimulated the incorporation of [^{3}H]glycerol 3-phosphate into neutral lipids by 2.9-fold, when palmitoyl-CoA was generated in the assay system. Insulin (20 mU/ml) and corticosterone (10^{-5} M) together only produced a 1.6-fold increase (table 1). There were no significant differences in neutral lipid formation when insulin (20 mU/ml) was added alone. Of the neutral lipids, 15–20% were triacylglycerols, and the remainder was mainly in the form of diacylglycerol. No significant difference in this figure could be found after hormonal treatments.

The incorporation of [^{3}H]glycerol 3-phosphate into all lipid classes was increased by 1.6-fold in the presence of 10^{-5} M corticosterone. The simultaneous presence of insulin (20 mU/ml) and corticosterone (10^{-5} M) reduced this increase to ~1.3-fold whilst the presence of insulin (20 mU/ml) alone had no effect upon total incorporation. Corticosterone (10^{-5} M) also increased [^{3}H]glycerol 3-phosphate incorporation into phospholipids (mainly phosphatidate) by 1.4-fold, whereas the presence of 20 mU insulin/ml and 10^{-5} M corticosterone together, or the presence of insulin (20 mU/ml) alone produced small decreases in phospholipid formation.

There were no significant changes in the maximum activities of acyl-CoA synthetase, microsomal and mitochondrial glycerol phosphate acyltransferase and diacylglycerol acyltransferase with any of the hormonal treatments when these activities were measured separately. We had expected that insulin would increase the glycerol phosphate acyltransferase (especially the mitochondrial activity) in view of [3,4]. The hepatocytes used in here were responsive to insulin since it antagonized the stimulating effects of corticosterone on the activity of phosphatidate phosphohydrolase ([8], table 1). Furthermore, insulin at 10–40 mU/ml stimulated the incorporation of $3H_2O$ into fatty acids by 34 ± 18% (mean ± SD) in 7 independent expt. (E.H. Mangiapane, D.N.B., unpublished). Possibly the major effect of insulin in stimulating the hepatic synthesis of triacylglycerols is by altering substrate availability rather than by changing

Table 1

Effects of insulin and corticosterone on the activities of some enzymes of triacylglycerol synthesis

Incorporation or enzyme activity	Control values after 6 h incubation without hormones (nmol product . min^{-1} . U lactate dehydrogenase^{-1})	Relative activity (%) in the presence of:		
		(I) 10^{-5} M corticosterone	(II) 20 mU insulin/ml	(III) 10^{-5} M corticosterone + 20 mU insulin/ml
(1) Total incorporation of [^{3}H]glycerol 3-phosphate into lipids	2.09 ±1.00 (7)	162±42 (7) I *vs* control[b]	109±46 (4)	128±66 (4)
(2) [^{3}H]Glycerol 3-phosphate incorporation into phospholipids	1.94 ±0.80 (6)	137±31 (6) I *vs* control[a]	88± 4 (3) II *vs* control[a]	88±11 (3)
(3) [^{3}H]Glycerol 3-phosphate incorporation into neutral lipids	0.320±0.283 (6)	286±104 (6) I *vs* control[c]	80±18 (3)	156±88 (3)
(4) Acyl-CoA synthetase	8.24 ±0.70 (3)	118±26 (3)	103±12 (3)	89± 8 (3)
(5) Total glycerol 3-phosphate acyltransferase	0.975±0.164 (5)	109±12 (5)	98± 8 (5)	91±19 (5)
(6) Mitochondrial glycerol 3-phosphate acyltransferase	0.701±0.107 (5)	106±15 (5)	85±18 (5)	87±16 (5)
(7) Microsomal glycerol 3-phosphate acyltransferase	0.273±0.074 (5)	116±18 (5)	101±39 (5)	106±40 (5)
(8) Phosphatidate phosphohydrolase	1.41 ±0.46 (4)	180±19 (4) I *vs* control[d]	90±10 (4)	118±12 (4)
(9) Diacylglycerol acyltransferase	0.345±0.106 (4)	97±11 (4)	94±11 (4)	106±26 (4)

Significant differences were determined by the paired *t*-test where: [a] $P < 0.05$; [b] $P < 0.02$; [c] $P < 0.01$; [d] $P < 0.001$

Hepatocytes were incubated with and without hormones for 6 h at 37°C [7]. Homogenates of the cells were prepared by sonication [7], divided into 6 equal portions and frozen in liquid N_2. Incorporations and enzyme assays were performed as in section 2. The incorporation of [^{3}H]glycerol 3-phosphate into various lipid fractions (1–3) refers to the incorporations of palmitate, in the presence of ATP, CoA and other cofactors which were optimized for that system. By contrast, glycerol phosphate acyltransferase activity (5–7) refers to the esterification using exogenous palmitoyl-CoA. The results are expressed as means ± SD with the number of experiments given in parentheses

the activities of enzymes directly involved in this synthesis.

Measurement of the incorporation of glycerol phosphate into total lipid when generating palmitoyl-CoA from palmitate should also theoretically be a measurement of the total glycerol phosphate acyltransferase activity. It is very unlikely that acyl-CoA synthetase is rate-limiting in the esterification process in mitochondrial [19], or microsomal fractions [15]. Insulin also failed to increase the rate of glycerol phosphate incorporation into total lipids in this system, but corticosterone did increase this rate by 62%. When this latter measurement was made using exogenous palmitoyl-CoA there may have been a small increase in activity (table 1), but this was not statistically significant and it was not in the region of 1.6-fold. However, the reaction conditions used in these two assays were also different in that the latter system did not include $MgCl_2$ [9]. The omission of $MgCl_2$ would decrease the activity of phosphatidate phosphohydrolase [20] and thus the conversion of phosphatidate to diacylglycerol.

The experiments shown in fig. 1 were performed

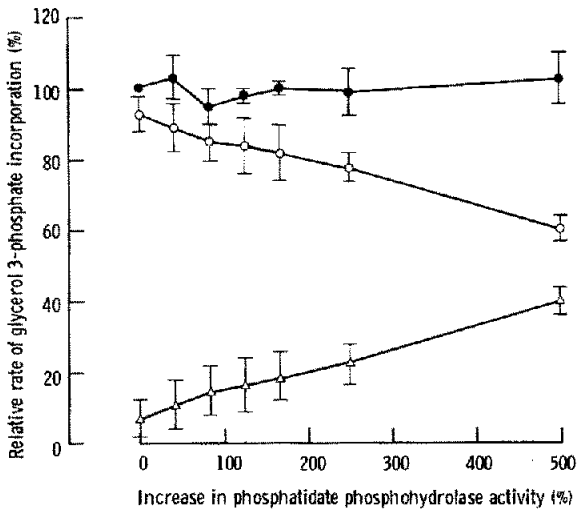

Fig.1. Effect of adding partially purified phosphatidate phosphohydrolase on the incorporation of [^{3}H]glycerol 3-phosphate into lipids. The incorporation into total lipids (•), phospholipids (○) and neutral lipids (△) by hepatocyte homogenates is shown as the mean ± SD of 3 independent expt. The values for the total lipids are given as a % of that obtained when no purified phosphatidate phosphohydrolase was added. Incorporations into phospholipids and neutral lipids are expressed as a % of the total incorporation. The activity of the phosphohydrolase in the homogenates and in the purified preparation were determined by using a mixed emulsion of phosphatidylcholine and [^{3}H]phosphatidate (section 2). The abscissa of the graph indicates the expected relative increase in the phosphohydrolase activity based on this determination.

to see whether the increase in phosphatidate phosphohydrolase activity, that is induced by corticosterone, could in itself explain the increase in lipid synthesis from glycerol phosphate. This might happen if the accumulation of phosphatidate in the membranes were to inhibit the further esterification of glycerol phosphate. Alternatively, conversion of phosphatidate to diacylglycerol might prevent a part of its degradation by phospholipase A activities [20]. However, the addition of phosphatidate phosphohydrolase that had been partially purified from the soluble fraction of rat liver did not increase the total esterification of glycerol phosphate. At present, we cannot explain why stimulations were produced by corticosterone in systems 1 and 2 of table 1, but not in system 5. The magnitude of the increase in neutral lipid formation that resulted from adding extra phosphatidate phosphohydrolase was very close to that predicted from the activity which was measured with a phosphatidate emulsion (fig.1). This further demonstrates that this method of analysis gives meaningful results in terms of the enzyme's ability to degrade membrane-bound phosphatidate.

In our previous work with hepatocytes we measured the effects of corticosterone and insulin on the total phosphatidate phosphohydrolase by using mixed emulsions of phosphatidate and phosphatidylcholine [7,8]. Corticosterone (10^{-5} M) increased this activity by 1.7–1.8-fold and this is confirmed in table 1 (system 8). This measurement includes the activities of a number of different phosphatidate phosphohydrolases which are present in the homogenates, some of which may not participate in glycerolipid synthesis. System (3) of table 1 gives an indirect measurement of the phosphohydrolase that was able to act on the membrane-bound phosphatidate that was newly synthesized from glycerol phosphate. As such, this should provide an estimate of the activity of the phosphohydrolase(s) that participate in glycerolipid synthesis. This activity was increased by 2.9- rather than by 1.8-fold after incubation with corticosterone and this effect was also partially reversed by insulin.

Phosphatidate phosphohydrolase was the only enzyme of those examined whose maximum velocity changed significantly after the hepatocytes had been incubated with corticosterone or insulin. We believe that the corticosterone-induced increase in the phosphohydrolase activity enables the liver to partially maintain its synthesis of phosphatidylcholine and phosphatidylethanolamine in times of stress. It also enables the liver to store excess fatty acids in triacylglycerols (as a fatty liver), or to secrete them as very low density lipoproteins in these conditions [7,8].

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REFERENCES

[1] McGarry, J.D. and Foster, D.W. (1980) Annu. Rev. Biochem. 49, 395–420.
[2] Bates, E.J. and Saggerson, E.D. (1977) FEBS Lett. 84, 229–232.
[3] Bates, E.J. and Saggerson, E.D. (1979) Biochem. J. 182, 751–762.
[4] Bates, E.J., Topping, D.L., Soorana, S.R. and Saggerson, E.D. (1977) FEBS Lett. 84, 225–228.
[5] Brindley, D.N. (1981) Clin. Sci. 61, 129–133.
[6] Lehtonen, M.A., Savolainen, M.J. and Hassinen, I.E. (1979) FEBS Lett. 99, 162–166.
[7] Jennings, R.J., Lawson, N., Fears, R. and Brindley, D.N. (1981) FEBS Lett. 133, 119–122.
[8] Lawson, N., Jennings, R.J., Fears, R. and Brindley, D.N. (1982) FEBS Lett. 143, 9–12.
[9] Lawson, N., Jennings, R.J., Pollard, A.D., Sturton, R.G., Ralph, S.J., Marsden, C.A., Fears, R. and Brindley, D.N. (1981) Biochem. J. 200, 265–273.
[10] Bates, E.J. and Saggerson, E.D. (1981) FEBS Lett. 128, 230–232.
[11] Lawson, N., Pollard, A.D., Jennings, R.J., Gurr, M.I. and Brindley, D.N. (1981) Biochem. J. 200, 285–294.
[12] Sturton, R.G., Pritchard, P.H., Han, L.-Y. and Brindley, D.N. (1978) Biochem. J. 174, 667–670.
[13] Polokoff, M.A. and Bell, R.M. (1978) J. Biol. Chem. 253, 7173–7178.
[14] Polokoff, M.A. and Bell, R.M. (1980) Biochim. Biophys. Acta 618, 129–142.
[15] Lloyd-Davies, K.A. and Brindley, D.N. (1975) Biochem. J. 152, 39–49.
[16] Hajra, A.K. (1968) J. Biol. Chem. 243, 3458–3465.
[17] Dodds, P.F., Gurr, M.I. and Brindley, D.N. (1976) Biochem. J. 160, 693–700.
[18] Brindley, D.N. and Bowley, M. (1975) Biochem. J. 148, 461–469.
[19] Sanchez, M., Nicholls, D.G. and Brindley, D.N. (1973) Biochem. J. 132, 697–706.
[20] Sturton, R.G. and Brindley, D.N. (1980) Biochim. Biophys. Acta 619, 494–505.