Both insulin and epidermal growth factor stimulate fatty acid synthesis and increase phosphorylation of acetyl-CoA carboxylase and ATP-citrate lyase in isolated hepatocytes

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Insulin and EGF cause identical stimulation (≃40%) of fatty acid synthesis in hepatocytes isolated from rats which have been starved and then refed a low-fat diet. In both cases this stimulation is associated with increased phosphorylation of ATP-citrate lyase and of a specific site on acetyl-CoA carboxylase. However, the altered phosphorylation of acetyl-CoA carboxylase is not associated with a change in kinetic parameters which is detectable in the purified enzyme. Whatever the mechanism involved, stimulation of fatty acid synthesis by growth factors may have a role in providing new phospholipid for growth of membranes.

Insulin *Epidermal growth factor* *ATP-citrate lyase* *Acetyl-CoA carboxylase* *Protein phosphorylation*

1. INTRODUCTION

Fatty acid synthesis is acutely regulated by several hormones. Glucagon in hepatocytes [1,2], and adrenaline [3] and glucagon [4] in adipocytes, cause inhibition of fatty acid synthesis, and lead to increased phosphorylation of the two lipogenic enzymes, ATP-citrate lyase and acetyl-CoA carboxylase [5–8]. Peptide mapping data suggest that these phosphorylations are catalyzed by cyclic AMP-dependent protein kinase [8–11], and the phosphorylation of acetyl-CoA carboxylase, but not ATP-citrate lyase, causes inactivation of the enzyme, sufficient to account for the inhibition of fatty acid synthesis by these hormones [12–14].

Insulin stimulates fatty acid synthesis in both adipocytes and hepatocytes, and, contrary to expectation, also leads to increased phosphorylation of both ATP-citrate lyase and acetyl-CoA carboxylase [2,15–18]. In the case of ATP-citrate lyase the insulin-stimulated phosphorylation occurs at the same serine residue as that phosphorylated by cyclic AMP-dependent kinase [18], while for acetyl-CoA carboxylase the insulin-stimulated site and the cyclic AMP-dependent protein kinase site are distinct [16,17]. Current evidence suggests that insulin acts by increasing the activity of a soluble, Ca^{2+} and cyclic AMP-independent protein kinase [19].

The insulin receptor shares a common feature with receptors for certain growth factors (e.g., EGF, PDGF, IGF-1) in that it possesses ligand-dependent tyrosine kinase activity [20–23]. Treatment of cultured cells with insulin or growth factors such as EGF also brings about increased phosphorylation, on serine residues, of the same set of intracellular proteins, including ribosomal protein S6 [24], and an unidentified, heat-stable 22-kDa polypeptide [25]. We therefore compared the effects of insulin and EGF on the rate of fatty acid synthesis and the phosphorylation of lipogenic enzymes in isolated hepatocytes.

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Abbreviations: EGF, epidermal growth factor; IGF-1, insulin-like growth factor-1; HPLC, high-performance liquid chromatography; PDGF, platelet-derived growth factor

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2. MATERIALS AND METHODS

Isolation of hepatocytes from rats which had been starved and then refed a low-fat diet, and measurement of fatty acid synthesis were as in [10]. Purification of acetyl-CoA carboxylase from hepatocytes, and estimation of its kinetic parameters and specific radioactivity were as in [10]. ^{32}P-labelled acetyl-CoA carboxylase was digested with trypsin and analysed by reversed-phase HPLC as in [17] except that an on-line radioactive monitor (Reeve Analytical Ltd, Glasgow) connected to a Hewlett-Packard 85B microcomputer was used to detect and quantify radioactive peaks. Purification of acetyl-CoA carboxylase from lactating rat mammary gland and its phosphorylation by casein kinase II was as in [14]. Casein kinase II was purified from rabbit skeletal muscle [26] and was a gift from J.R. Woodgett of this department. EGF purified from mouse submaxillary gland was a gift from Dr Peter Parker of the Imperial Cancer Research Fund, London. Porcine insulin was from Novo Industries (Denmark). Sources of other materials are as in [10,14].

3. RESULTS

3.1. *Effects of insulin and EGF on fatty acid synthesis*

As reported previously [2], insulin causes a modest stimulation of fatty acid synthesis in isolated hepatocytes (table 1). Maximal effects are observed at >10 nM insulin, with half-maximal effects at 0.8 nm (not illustrated). We now report that EGF produces an almost identical effect to that of insulin (table 1). Maximal effects were observed at >10 nM EGF (not shown).

3.2. *Effects of insulin and EGF on the phosphorylation of cytosolic proteins*

Isolated hepatocytes were preincubated with [^{32}P]phosphate for 60 min to achieve steady-state labelling of acetyl-CoA carboxylase and other proteins [10,27] and were then treated with or without insulin or EGF for 15 min. Fig.1 shows that phosphorylation of 116- and 46-kDa polypeptides was stimulated by both insulin and EGF, as found by others (unpublished data quoted in [28]). The 116-kDa phosphopeptide is not observed in cells from fasted rats (not illustrated), comigrates with purified ATP-citrate lyase, and has been identified as this latter protein previously [29]. The effects of EGF on the phosphorylation of these two polypeptides are smaller than those of insulin (fig.1), particularly for the 46-kDa polypeptide, although maximally effective doses were used for each hormone/growth factor. The 240-kDa phosphopeptide, identified as acetyl-CoA carboxylase [5,10],

Table 1

Effects of insulin and EGF treatment of isolated hepatocytes on rates of fatty acid synthesis and on the properties of acetyl-CoA carboxylase purified from the cells

	Control	Insulin (1 mU/ml)	EGF (100 ng/ml)
Pathway parameter			
Rate of fatty acid synthesis (relative to control)	1.0	1.37 ± 0.04 (15)[b]	1.36 ± 0.05 (4)[a]
Enzyme parameters			
V_{max} (μmol/min per mg)	317 ± 58 (5)	274 ± 35 (5)	312 ± 65 (3)
V_{max} (relative to control)	1.0	0.90 ± 0.07 (5)	0.90 ± 0.07 (3)
K_a for citrate (mM)	7.5 ± 1.0 (5)	7.6 ± 0.9 (5)	7.0 ± 0.3 (3)
Specific radioactivity (relative to control)	1.0	0.99 ± 0.04 (9)	0.99 ± 0.03 (6)

Rates of fatty acid synthesis were measured using [^{3}H]H_2O [10] between 5 and 35 min after hormone addition. Specific radioactivity of acetyl-CoA carboxylase was determined for enzyme isolated from ^{32}P-labelled cells [10]. Data are expressed as mean ± SE of the mean for the number of cell incubations shown in brackets. Data shown with asterisks are significantly different from controls by the paired *t*-test: [a] $p < 0.01$; [b] $p < 0.001$

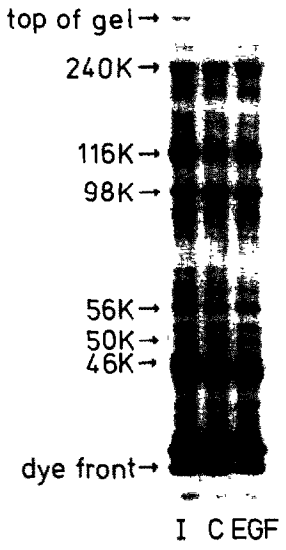

Fig.1. Analysis of radioactivity in proteins in a 100000 × *g* supernatant fraction prepared from ^{32}P-labelled hepatocytes. Proteins were separated in a 7% polyacrylamide gel in the presence of SDS and were detected by autoradiography as in [10]. The molecular masses of major phosphoproteins (shown at left) were estimated by reference to the mobility of marker proteins as in [10]. I, insulin (0.8 nM); C, control; EGF, EGF (16 nM).

showed no apparent change in total ^{32}P-labelling in response to insulin or EGF (fig.1).

3.3. *Effects of insulin and EGF on the properties of purified acetyl-CoA carboxylase*

Isolated hepatocytes were preincubated with [^{32}P]phosphate for 60 min and then acetyl-CoA carboxylase was purified from control, insulin- and EGF-treated cells by avidin-Sepharose chromatography. There were no significant changes in the specific radioactivity or the kinetic parameters of the isolated enzyme (table 1). However, when tryptic digests were analysed by reversed-phase HPLC, both insulin (fig.2, table 2) and EGF (table 2) caused increases in the ^{32}P-labelling of a specific tryptic peptide (peptide 4). This peptide was distinct from that containing the major site of phosphorylation for cyclic AMP-dependent protein kinase (which corresponded to peptide 1; not illustrated), but comigrated with the peptide containing the major site of phosphorylation for casein kinase II, both on reversed-phase

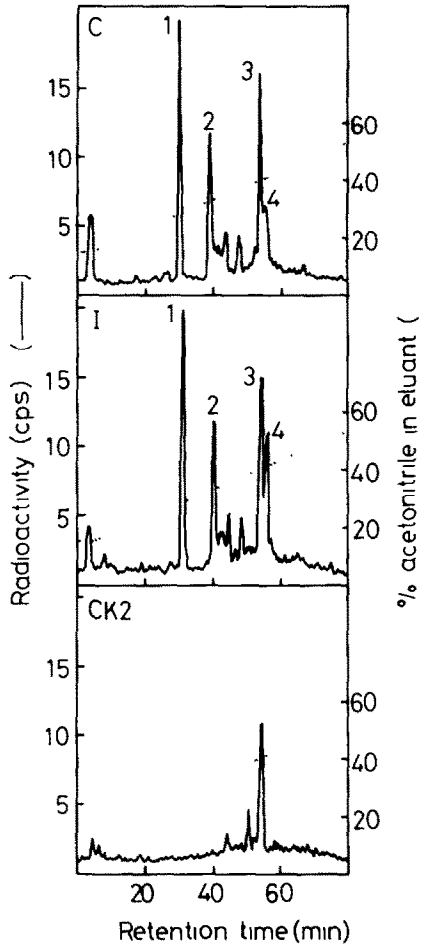

Fig.2. Analysis of radioactivity in tryptic peptides of purified acetyl-CoA carboxylase. C, enzyme purified from control ^{32}P-labelled cells; I, enzyme purified from insulin-treated (0.8 nM) cells; CK2, enzyme purified from mammary gland and phosphorylated in vitro with [γ-^{32}P]ATP and casein kinase II.

HPLC (fig.2) and thin-layer isoelectric focussing. The ^{32}P-labelling of peptide 1 was unchanged by insulin or EGF; labelling of peptide 3 did not change significantly with insulin but decreased slightly with EGF (table 2). Peptide 2 was not observed consistently and appears to be related to peptide 4, since on some occasions digestion of acetyl-CoA carboxylase phosphorylated by casein kinase II gave a peptide with the same retention time as peptide 2 (not illustrated). Peptides 2 and 4 were therefore counted together for the quantification in table 2.

Table 2

Effects of insulin and EGF treatment on the ^{32}P-labelling of individual tryptic peptides of acetyl-CoA carboxylase

	Control	Insulin (1 mU/ml)	EGF (100 ng/ml)
Peak 1	1.0	0.97 ± 0.03 (4)	1.00 ± 0.06 (4)
Peak 3	1.0	0.99 ± 0.09 (4)	0.85 ± 0.02 (4)[b]
Peak 4 (+ 2)	1.0	1.39 ± 0.03 (4)[c]	1.92 ± 0.22 (4)[a]

Results are expressed relative to control incubations. Peaks were quantified after separations similar to those in fig.2, as described in section 2. Peaks 2 and 4 have been counted together as discussed in section 3. Data marked are significantly different from controls by the paired *t*-test: [a] $p < 0.01$; [b] $p < 0.01$; [c] $p < 0.002$

4. DISCUSSION

These results emphasize the similarity between the acute effects of insulin and those of growth factors such as EGF. Both extracellular messengers stimulate autophosphorylation of their respective cell surface receptors on tyrosine residues [28]; both cause increased phosphorylation on serine residues of the same set of intracellular proteins [24,25] to which may now be added ATP-citrate lyase and acetyl-CoA carboxylase. We also now report that insulin and EGF have identical effects on the rate of fatty acid synthesis in isolated hepatocytes. Despite this correlation between increased phosphorylation of lipogenic enzymes and increased fatty acid synthesis, it is still not clear that the two events are causally related. Insulin treatment of adipocytes [19] or hepatocytes [2] leads to increases in acetyl-CoA carboxylase activity measurable in crude extracts of the cells. However, while the effect of insulin on phosphorylation of acetyl-CoA carboxylase in adipocytes survives purification of the enzyme, the effect on enzyme activity does not [17]. Our present results show that this dissociation of the effects on activity and phosphorylation also holds for effects of both insulin and EGF in hepatocytes. These findings imply either that the change in phosphorylation is unrelated to enzyme activation, or that a factor which is removed during enzyme purification is necessary for the effect of phosphorylation at the insulin- and EGF-sensitive site to be observed.

The correspondence between the insulin- and EGF-sensitive peptide on acetyl-CoA carboxylase and a peptide containing the major site of phosphorylation by casein kinase II is in agreement with results obtained previously on insulin treatment of adipocytes [17]. Phosphorylation within this peptide by casein kinase II has no effect on the activity of purified acetyl-CoA carboxylase [14,30]. However, although comigration in two different analytical systems suggests strongly that the peptides phosphorylated by casein kinase II and in response to insulin/EGF are identical, it remains to be demonstrated whether the same residue within this peptide is phosphorylated in each case. Witters et al. [17] also reported that casein kinase I phosphorylates a peptide similar in retention time to the insulin-sensitive peptide, and Cobb and Rosen [31] have reported that a protein kinase phosphorylating ribosomal protein S6, and which is enhanced in activity following insulin treatment of 3T3-L1 cells, resembles casein kinase I. We found that casein kinase I did phosphorylate site(s) on acetyl-CoA carboxylase in a peptide similar in retention time to the insulin- and EGF-sensitive peptide, but that it phosphorylated numerous other sites also, and hence lacks the specificity to account for the effects of insulin and EGF (not shown).

The precise physiological role of EGF is still far from clear, so the significance of the stimulation of fatty acid synthesis by EGF is currently difficult to evaluate. Of the three identified intracellular protein phosphorylations which are stimulated by insulin and growth factors, two (ATP-citrate lyase and acetyl-CoA carboxylase) are associated with stimulation of fatty acid synthesis and one (ribosomal protein S6) with activation of protein synthesis. It is not unreasonable that rapidly growing and/or dividing cells would require rapid fatty acid synthesis for the supply of membrane lipid, just as they will require new protein. Although the activation of fatty acid synthesis may be a rather peripheral event in the overall effect of growth factors on cell growth and division, it does represent a simple system with which to attempt elucidation of the mechanism of growth factor action. In particular, it may prove useful in the identification of

protein (serine/threonine) kinases which are activated by growth factors. Further studies are being conducted along this approach.

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