Epidermal growth factor mimics insulin effects in rat hepatocytes

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Epidermal growth factor (EGF) mimicked the effect of insulin to activate glycogen synthase and stimulate glycogen synthesis in isolated rat hepatocytes. Both agents required glucose (> 5 mM) and had similar time courses of action. The maximum effect of EGF was approx. 70% of that of insulin, and the half-maximally effective concentrations were 9 nM and 4 nM respectively. Combinations of the two agents produced additive responses. EGF also resembled insulin in its ability to inhibit the effects of 0.1–1.0 nM-glucagon on cyclic AMP and glycogen phosphorylase in hepatocytes. The maximum effect of EGF was approx. 70% of that of insulin, and the half-maximally effective concentrations were approx. 5 nM and 0.5 nM respectively. EGF and insulin inhibited phosphorylase activation by exogenous cyclic AMP, and inhibited cyclic AMP accumulation induced by forskolin. They also inhibited phosphorylase activation provoked by phenylephrine, but not by vasopressin. EGF added alone rapidly activated phosphorylase and increased cytosolic $[Ca^{2+}]$, but the effects were no longer apparent at 5 min and were smaller than those of vasopressin. Insulin did not induce these changes. In hepatocytes previously incubated with *myo*- $[^3H]$ inositol, EGF did not significantly increase *myo*-inositol 1,4,5-trisphosphate. However, its ability to increase cytosolic $[Ca^{2+}]$ was blocked by neomycin, an inhibitor of phosphatidylinositol bisphosphate hydrolysis. It is concluded that some, but not all, of the effects of EGF in liver are strikingly similar to those exerted by insulin, suggesting that these agents may have some similar mechanisms of action in this tissue.

INTRODUCTION

Epidermal growth factor (EGF) and insulin share many features. They are both polypeptides which stimulate growth and are internalized in their target tissues. Both their receptors exhibit tyrosine kinase activity, which is stimulated by binding of the respective polypeptide (for reviews, see Czech, 1985; Pike & Krebs, 1986). Their receptor kinases show many similarities, but do not have identical substrate specificities and are not equally sensitive to inhibition by *N*-ethylmaleimide (Pike *et al.*, 1984; Klein *et al.*, 1985; Uhing & Exton, 1986). Furthermore, they differ in their Mg^{2+} and Mn^{2+} specificities in certain tissues (Carpenter *et al.*, 1979; Uhing & Exton, 1986; cf. Pike *et al.*, 1984).

Despite the presence of both EGF and insulin receptors in liver (Freychet *et al.*, 1971; O'Keefe *et al.*, 1974; Cohen *et al.*, 1982) and other tissues, few studies have compared the effects of EGF and insulin in these tissues. For example, Fehlmann *et al.* (1981) have reported that EGF, like insulin (Fehlmann & Freychet, 1981), stimulates Na^+ influx into hepatocytes. However, it has no effect on amino acid transport, but inhibits the stimulatory effect of glucagon (Morin *et al.*, 1981). On the other hand, insulin stimulates amino acid transport and has an additive effect with glucagon (Fehlmann *et al.*, 1979). Holland & Hardie (1985) have shown that EGF and insulin cause identical stimulation of fatty acid synthesis in hepatocytes from re-fed rats. This is associated with increased phosphorylation of both ATP citrate lyase and acetyl-CoA carboxylase. Vaartjes *et al.* (1985) have confirmed the stimulation of fatty acid synthesis by both agents.

Diamond *et al.* (1978) have demonstrated that both

EGF and insulin stimulate glycolysis in 3T3 cells, and Chan & Krebs (1985) have reported that EGF mimics insulin in its stimulatory effects on glycogen synthase activity in these cells. In contrast, EGF slowly activates glycogen synthase in A431 cells, but these cells show no response to insulin (Chan & Krebs, 1985). In the present study, we have examined the ability of EGF to mimic the ability of insulin to stimulate glycogen synthase and inhibit glucagon and α_1 -adrenergic effects in rat hepatocytes.

EXPERIMENTAL

Materials

EGF (from mouse submaxillary glands), cyclic AMP, quin 2 acetoxymethyl ester, (–)-phenylephrine hydrochloride and [8-arginine]vasopressin were from Sigma. Forskolin was from Calbiochem. Insulin and glucagon were gifts from Eli Lilly Co. UDP-[U- ^{14}C]glucose and [U- ^{14}C]glucose 1-phosphate were from ICN. D-[U- ^{14}C]Glucose was from New England Nuclear. Collagenase (type 1) was from Cooper Biomedical. Other reagents were of the highest purity commercially available.

Methods

Hepatocytes were prepared from fed male rats (200–250 g body wt.) as described in detail elsewhere (Blackmore & Exton, 1985). Glycogen synthase *a* activity was assayed as described by Strickland *et al.* (1980), and [^{14}C]glucose incorporation into glycogen was determined as described by Chan & Exton (1976). Phosphorylase *a* activities and $[Ca^{2+}]_i$ were measured as

Table 1. Effect of EGF and insulin on glycogen synthase activity and [14 C]glycogen synthesis from [14 C]glucose in hepatocytes

For glycogen synthase measurements, hepatocytes from fed rats were incubated with 30 mM unlabelled glucose in the presence or absence of 6 nM-insulin or 7 nM-EGF, and glycogen synthase *a* was assayed after 20 min. Values are means $\pm$ S.E.M. for four experiments. For glycogen-synthesis measurements, hepatocytes were incubated with 30 mM-D-[14 C]-glucose for 60 min with or without 6 nM-insulin or 7 nM-EGF. [14 C]Glycogen formation was measured as described by Chan & Exton (1976) and is expressed as a percentage of control with 30 mM-[14 C]glucose alone. Data are means $\pm$ S.E.M. from sextuplicate assays in a representative experiment.

Experimental condition	Glycogen synthase <i>a</i> (% increase above glucose control)	[14 C]Glycogen (% increase above glucose alone)
EGF	21 $\pm$ 4	28 $\pm$ 4
Insulin	35 $\pm$ 2	61 $\pm$ 14
EGF + insulin	53 $\pm$ 1	92 $\pm$ 8

described by Blackmore & Exton (1985), and cyclic AMP was measured by the method of Steiner *et al.* (1969) as modified by Harper & Brooker (1975) in HClO_4 extracts of hepatocytes (Morgan *et al.*, 1983).

Hepatocytes were labelled for 90 min with *myo*-[^3H]inositol as previously described (Charest *et al.*, 1985), and the inositol phosphate esters were chromatographed on a Whatman Partisil SAX-10 h.p.l.c. column as described by Batty *et al.* (1985) and Irvine *et al.* (1985).

RESULTS

Effects of EGF and insulin on glycogen synthase in hepatocytes

Table 1 shows that EGF and insulin alone or in combination activate glycogen synthase and stimulate the incorporation of [14 C]glucose into glycogen in hepatocytes. The effects are detectable at 2 min and maximal at 10 min and require the presence of glucose (> 5 mM) (results not shown). The maximal effect of EGF on glycogen synthase (measured in the presence of 30 mM-glucose) was approx. 70% of that of insulin, and half-maximal activations were seen with 9.0 ± 0.8 nM-EGF and 4.4 ± 1.1 nM-insulin (Fig. 1). Combination of increasing concentrations of EGF with a fixed concentration of insulin (6 nM) produced a strictly additive effect on glycogen synthase *a* (Fig. 2). The largest increase was greater than that produced by a maximally effective concentration (200 nM) of insulin alone and was not significantly different from that produced by combining maximally effective concentrations of both agents (Fig. 2, $\square$ and $\triangle$). Similar results were obtained if increasing concentrations of insulin were combined with a fixed concentration of EGF (results not shown). Total glycogen synthase, i.e. that assayed in the presence of 10 mM-glucose 6-phosphate, was also assayed in the above experiments. The data are not shown since total glycogen synthase activity always changed in parallel with glycogen synthase *a*, as observed by previous workers (Strickland *et al.*, 1980; Thomas *et al.*, 1985).

Fig. 1. Dose-response curves for the activation of glycogen synthase by insulin and EGF

Hepatocytes from fed rats were incubated for 20 min with 30 mM-glucose and the concentrations of insulin and EGF shown. The changes in glycogen synthase *a* are plotted as percentage increases above control with 30 mM-glucose alone. Data are means $\pm$ S.E.M. from two experiments per agent, assayed in quadruplicate.

Fig. 2. Additivity of EGF and insulin effects on glycogen synthase

Hepatocytes from fed rats were incubated for 20 min with 30 mM-glucose and different concentrations of EGF in the presence or absence of 6 nM-insulin. Glycogen synthase *a* values are given as percentage increases above control with 30 mM-glucose alone. Also shown are the values with a maximally effective concentration of insulin (200 nM) and with 200 nM-insulin plus maximally effective EGF (200 nM). The data are means $\pm$ S.E.M. from three experiments, assayed in quadruplicate.

Effects of EGF and insulin on glucagon actions

The ability of EGF to mimic insulin inhibition of glucagon actions was next tested. Fig. 3 illustrates that prior incubation of hepatocytes with EGF or insulin for 10 min or 2 min (results not shown) inhibited the activation of phosphorylase and accumulation of cyclic

Fig. 3. Time course of glucagon (0.5 nM) effects on cyclic AMP concentrations and phosphorylase *a* activity in the presence and absence of EGF (33 nM) or insulin (6 nM)

Hepatocytes (10 ml of hepatocyte suspension in 150 ml polycarbonate Erlenmeyer flasks) were preincubated in the presence and absence of EGF or insulin for 10 min before glucagon was added. Duplicate 0.5 ml samples were removed at the appropriate times for the measurement of cyclic AMP and phosphorylase *a*. One sample was added to plastic test tubes immersed in liquid N_2 for the measurement of phosphorylase, and the other was added to 0.5 ml of 10% $HClO_4$ for cyclic AMP determination. The values shown are means $\pm$ S.E.M. from triplicate incubations, assayed in duplicate. Results of a representative experiment of three are shown.

AMP induced by 0.5 nM-glucagon. The concentrations of EGF and insulin that half-maximally inhibited the actions of 0.5 nM-glucagon were approx. 5 and 0.5 nM respectively (results not shown). In the absence of glucagon, neither EGF nor insulin had significant effects on phosphorylase *a* or cyclic AMP measured at 10 min (Figs. 3 and 4), but see below. Both agents were effective against 0.1–1.0 nM-glucagon (Fig. 4a), but at higher concentrations of glucagon the effect was progressively diminished (results not shown). When maximally effective concentrations of EGF and insulin were combined, the inhibition of glucagon action was no greater than that observed with insulin alone (results not shown).

Ca^{2+} depletion of the hepatocytes decreased the basal phosphorylase *a* activity (Fig. 4b), but did not significantly alter the fold increases in phosphorylase *a* or cyclic AMP induced by different concentrations of glucagon. The

ability of EGF and insulin to inhibit either glucagon-induced phosphorylase activation or cyclic AMP accumulation was also not altered by Ca^{2+} depletion (Fig. 4b).

Effects of EGF and insulin on cyclic AMP and forskolin actions

Since the effects of EGF and insulin on phosphorylase *a* and cyclic AMP concentrations may be exerted on or before adenylate cyclase or on cyclic AMP phosphodiesterase, both agents were tested against exogenous cyclic AMP. In agreement with other reports (Blackmore *et al.*, 1979; Beebe *et al.*, 1985), Fig. 5 shows that insulin inhibited phosphorylase activation induced by exogenous cyclic AMP and that EGF was almost as effective. When tested against 5–20 μ M-forskolin, which activates adenylate cyclase by a non-receptor-mediated mechanism (Seamon & Daly, 1981; Insel *et al.*, 1982; Darfler *et al.*, 1982), both EGF and insulin decreased cyclic AMP accumulation and phosphorylase activation (results not shown).

Effects of EGF and insulin on α_1 -adrenergic action

Not only does insulin inhibit cyclic AMP-mediated actions in liver, but it is effective against α_1 -adrenergic agonists, but not vasopressin (Massague & Guinovart, 1978; Blackmore *et al.*, 1979; Strickland *et al.*, 1980; Dehaye *et al.*, 1981; Thomas *et al.*, 1985). Fig. 6(a) illustrates that EGF likewise inhibited phosphorylase activation induced by the specific α_1 -adrenergic agonist phenylephrine, but not that induced by vasopressin (Fig. 6b).

Rapid effects of EGF on hepatocytes

During the course of the present work, we became aware that another group had observed rapid effects of EGF on $[Ca^{2+}]_i$ in hepatocytes (Johnson *et al.*, 1986). In agreement with that report, Fig. 7 illustrates that EGF caused a rapid, dose-dependent, increase in $[Ca^{2+}]_i$ measured in quin2-loaded hepatocytes. These increases were associated with corresponding changes in phosphorylase *a* (results not shown). The increase in $[Ca^{2+}]_i$ induced by EGF was still observed, although to a lesser extent, when extracellular Ca^{2+} was chelated with EGTA (Fig. 8a). The ability of vasopressin to elevate $[Ca^{2+}]_i$ was also affected by EGTA treatment, but mainly after the initial increase had occurred. These results suggest that vasopressin and EGF elevate $[Ca^{2+}]_i$ initially by mobilizing intracellular Ca^{2+} . Extracellular Ca^{2+} is required after the initial intracellular Ca^{2+} mobilization has occurred (Charest *et al.*, 1985). In contrast with EGF, insulin was without effect on basal $[Ca^{2+}]_i$ or phosphorylase at any time point (results not shown), in agreement with previous reports (e.g. Blackmore *et al.*, 1979; Thomas *et al.*, 1985).

The increase in $[Ca^{2+}]_i$ induced by EGF was smaller and of slower onset than that induced by vasopressin and declined rapidly to reach basal values between 5 and 10 min (Fig. 7). This latter observation accounts for the fact that phosphorylase *a* values were basal in the presence of EGF in Figs. 3–6, since the measurements were made 10–15 min after addition of the agent. The data in Fig. 6 show that both insulin and EGF were able to inhibit the action of phenylephrine to activate phosphorylase. Consistent with this inhibition, the data in Fig. 8(b) show that EGF and insulin inhibited the ability of 0.2 μ M-phenylephrine to raise $[Ca^{2+}]_i$. Insulin

Fig. 4. Dose-response of glucagon to activate phosphorylase and increase cyclic AMP in the presence and absence of EGF (33 nM) or insulin (6 nM) in normal and Ca^{2+} -depleted hepatocytes

Hepatocytes were prepared normally (a) or depleted of Ca^{2+} by incubating them for 15 min in Krebs-Henseleit bicarbonate buffer to which no Ca^{2+} was added and to which 0.5 mM-EGTA was added (b). They were preincubated for 10 min with EGF or insulin before glucagon was added. Each incubation with glucagon was 2 ml total volume. Samples were removed after 5 min of incubation with glucagon for the measurement of cyclic AMP and phosphorylase (see legend to Fig. 3). Each value shown is the mean of triplicate incubations assayed in duplicate. The S.E.M. bars are omitted for the sake of clarity. Each panel shows a representative of three experiments. ○, Glucagon alone; ●, glucagon plus 33 nM-EGF; △, glucagon plus 6 nM-insulin.

and EGF were unable to inhibit the increase in $[\text{Ca}^{2+}]_i$ induced by vasopressin (0.01–1 nM) (results not shown).

In order to determine whether the ability of EGF to increase $[\text{Ca}^{2+}]_i$ was secondary to the formation of *myo*-inositol 1,4,5-trisphosphate, hepatocytes were incubated for 90 min with *myo*- ^3H inositol and then with EGF (33 nM). The production of *myo*- ^3H inositol 1,4,5-trisphosphate (separated from *myo*- ^3H inositol 1,3,4-trisphosphate) at 15 s, 30 s and 1 min was measured by h.p.l.c. (Batty *et al.*, 1985). At no time was there a significant increase in this inositol polyphosphate (results not shown). Neomycin has been used to inhibit agonist-induced phosphatidylinositol 4,5-bisphosphate hydrolysis in many systems (e.g. Carney *et al.*, 1985). The data in Fig. 8(c) show that treatment of hepatocytes with

2 mM-neomycin abolished the increase in $[\text{Ca}^{2+}]_i$ induced by EGF and vasopressin, thus implying a role for phosphatidylinositol 4,5-bisphosphate hydrolysis in the actions of both agents to increase $[\text{Ca}^{2+}]_i$. Neomycin (1–5 mM) by itself had no effect on basal $[\text{Ca}^{2+}]_i$.

DISCUSSION

The results of the present study illustrate that EGF closely mimics some of the actions of insulin in liver. It is known that liver possesses receptors for both insulin and EGF (Freychet *et al.*, 1971; O'Keefe *et al.*, 1974; Cohen *et al.*, 1982) and that these receptors exhibit tyrosine kinase activity (Cohen *et al.*, 1982; Rubin *et al.*, 1982; Kasuga *et al.*, 1982; Ehrhart *et al.*, 1983; Van

Fig. 5. Dose-response of exogenous cyclic AMP to activate phosphorylase in the presence and absence of EGF (33 nM) or insulin (6 nM)

Each value is the mean $\pm$ S.E.M. from triplicate incubations assayed in duplicate. Results of a representative experiment of two are shown. See the legend to Fig. 4 for other details. $\circ$, Cyclic AMP alone; $\bullet$, cyclic AMP plus 33 nM-EGF; $\triangle$, cyclic AMP plus 6 nM-insulin.

Obberghen *et al.*, 1983). However, only recently have the hepatic receptor kinases and effects of EGF and insulin been compared (Fehlmann *et al.*, 1981; Morin *et al.*, 1981; Klein *et al.*, 1985; Holland & Hardie, 1985; Vaartjes *et al.*, 1985; Uhing & Exton, 1986). These

studies have shown considerable similarities, but also some differences, in the kinases (Klein *et al.*, 1985; Uhing & Exton, 1986) and in the effects of EGF and insulin on amino acid transport (Fehlmann *et al.*, 1979; Morin *et al.*, 1981).

A key question to be addressed is whether or not the similar effects of EGF and insulin in liver are due to some common mechanism(s) of action. The effect of insulin on glycogen synthase is markedly enhanced by glucose (Witters & Avruch, 1978; Thomas *et al.*, 1985) and can be seen in the absence of agents that increase cyclic AMP (Miller & Larner, 1973; Witters & Avruch, 1978; Ciudad *et al.*, 1981; Thomas *et al.*, 1985). There is also evidence that it can occur without any detectable change in cyclic AMP concentration (Exton *et al.*, 1971; Miller & Larner, 1973; Pilgis *et al.*, 1975; Van de Werve *et al.*, 1977; Blackmore *et al.*, 1979). The present study shows that EGF likewise requires glucose to activate glycogen synthase and does not alter basal cyclic AMP. Thus its action might also involve activation of a protein phosphatase or inhibition of a cyclic AMP-independent protein kinase.

On the other hand, insulin can inhibit glucagon-stimulated cyclic AMP accumulation (for references, see Blackmore *et al.*, 1979), and this effect does not require exogenous glucose. There is much evidence that insulin decreases cyclic AMP in liver by stimulating a low- K_m form of cyclic AMP phosphodiesterase (Loten *et al.*, 1978; Heyworth *et al.*, 1984), whereas there is dispute as to whether or not insulin inhibits adenylate cyclase (Rosselin & Freychet, 1973). The question concerning additional effects of insulin on liver cyclic AMP-dependent protein kinase remains controversial (Miller & Larner, 1973; Van de Werve *et al.*, 1977; Morin *et al.*, 1981; Gabbay & Lardy, 1984; Beebe *et al.*, 1985).

Although the present study illustrates the close similarity between the effects of insulin and EGF to activate glycogen synthase and to inhibit cyclic AMP accumulation in liver, it also shows that insulin does not share the ability of EGF to increase $[Ca^{2+}]_i$ transiently. This difference is not surprising, since it is probable that

Fig. 6. Dose-response of phenylephrine (a) and vasopressin (b) to activate phosphorylase in the presence and absence of EGF (33 nM) or insulin (6 nM)

Each value is the mean of duplicate determinations from triplicate flasks. Results of a representative of two experiments are shown. See the legend of Fig. 4 for other details. $\circ$, Phenylephrine alone; $\bullet$, phenylephrine plus 33 nM-EGF; $\triangle$, phenylephrine plus 6 nM-insulin.

Fig. 7. Effect of vasopressin (10 nM) and EGF (various doses) on $[Ca^{2+}]_i$

Hepatocytes were loaded with quin2 as previously described (Blackmore & Exton, 1985), washed once and resuspended in medium containing 2.5 mM- Ca^{2+} . The basal $[Ca^{2+}]_i$ was approx. 200 nM, which increased to approx. 1100 nM with 10 nM-vasopressin and to approx. 500 nM with 30 nM-EGF, 0.5 min after hormone injection, which was made at 0.5 min. Each trace is the mean of either duplicate or triplicate experiments (Blackmore & Exton, 1985). The effects of other concentrations of EGF (given in nM) are shown. The numbers on the left-hand ordinate represent relative changes in fluorescence.

Fig. 8. Effect of extracellular Ca^{2+} on ability of EGF and vasopressin to raise $[Ca^{2+}]_i$ (a), effect of EGF and insulin to inhibit phenylephrine actions on $[Ca^{2+}]_i$ (b) and the effect of neomycin to inhibit vasopressin and EGF effects on $[Ca^{2+}]_i$ (c)

Hepatocytes were loaded with quin2 (Blackmore & Exton, 1985). In (a), EGF and vasopressin were added at 0.5 min to cells suspended in normal (2.5 mM)- Ca^{2+} -containing media or in normal- Ca^{2+} -containing media to which 3.5 mM-EGTA was added 1.0 min before the addition of EGF or insulin. In (b), cells were preincubated for 10 min with either EGF or insulin before a submaximal dose of phenylephrine (0.2 μ M) was added at 0.5 min. In (c), 2 mM-neomycin (Sigma) was added 0.5 min before the addition of vasopressin or EGF. Each trace shown (a, b and c) is the average of at least three separate incubations. Abbreviations: VASO, vasopressin; INS, insulin; PHENYL, phenylephrine; NEO, neomycin.

EGF and insulin have multiple mechanisms of action subsequent to receptor activation and that only some of these mechanisms are common to both agonists. An alternative explanation is that there are subtypes of EGF and insulin receptors, as found for many other receptors. Clearly, EGF does not mimic some of the classic effects of insulin in certain tissues, and vice versa.

The small effect of EGF to elevate $[Ca^{2+}]_i$ and activate phosphorylase in hepatocytes confirms the findings of Johnson *et al.* (1986). The increase in $[Ca^{2+}]_i$ appears to be due to the initial mobilization of internal Ca^{2+} stores, since it was not abolished by brief chelation of extracellular Ca^{2+} with EGTA (the present work; Johnson *et al.*, 1986). This is different from the situation in A431 cells, where the increase in $[Ca^{2+}]_i$ induced by EGF is dependent on extracellular Ca^{2+} (Moolenaar *et al.*, 1986).

Efforts to demonstrate an increase in *myo*-inositol 1,4,5-trisphosphate in $[^3H]$ inositol-labelled hepatocytes were unsuccessful. This suggests that EGF either acts on a poorly labelled pool of phosphatidylinositol 4,5-bisphosphate or elevates $[Ca^{2+}]_i$ by another mechanism. The former explanation receives some support from the observation that EGF stimulates the incorporation of $[^32P]P_i$ and *myo*- $[^3H]$ inositol into phosphatidylinositol in A431 cells (Sawyer & Cohen, 1981). The ability of EGF to increase $[Ca^{2+}]_i$ in hepatocytes was inhibited by incubating cells in the presence of 2 mM-neomycin (Fig. 8c), which has been found to inhibit agonist-stimulated *myo*-inositol 1,4,5-trisphosphate formation in fibroblasts (Carney *et al.*, 1985) and *myo*-inositol 1,4,5-trisphosphate-induced Ca^{2+} release in permeabilized liver cells (Prentki *et al.*, 1986). These data suggest that EGF does indeed mobilize intracellular Ca^{2+} by increasing *myo*-inositol 1,4,5-trisphosphate, but that our h.p.l.c. methods were unable to detect the increase. Previous studies indicate that maximal mobilization of intracellular Ca^{2+} by agonists is observed with only an approx. 20% increase in this ester (Charest *et al.*, 1985; Lynch *et al.*, 1985).

In agreement with previous reports (Blackmore *et al.*, 1979; Hobson *et al.*, 1980), the present study (Fig. 4) shows that Ca^{2+} depletion of hepatocytes does not modify the ability of insulin to decrease cyclic AMP and phosphorylase *a* in the presence of glucagon. The same is true for EGF (Fig. 4). Thus Ca^{2+} ions do not seem to be required for the actions of insulin or EGF on cyclic AMP concentrations in liver. As noted above, unlike EGF, insulin was without effect on total cell Ca^{2+} or cytosolic Ca^{2+} in hepatocytes (Blackmore *et al.*, 1979; Thomas *et al.*, 1985; the present work) and does not alter basal Ca^{2+} fluxes in the perfused rat liver (Dehay *et al.*, 1981).

The search for common features between EGF and insulin actions in liver and other tissues could provide some insights into the mechanisms of action of insulin. In particular, in view of the growing evidence that the tyrosine kinase activity of the insulin receptor plays an essential role (for references, see Morgan *et al.*, 1986), the search for common cellular substrates of the insulin- and EGF-receptor kinases might provide some clues.

Several proteins have been reported to be rapidly phosphorylated on tyrosine in cells treated with EGF (Hunter & Cooper, 1985). However, the functions of these proteins, apart from the EGF receptor and lipocortin (Pepinsky & Sinclair, 1986), are at present unknown, and it is uncertain whether or not their

phosphorylations are linked to the physiological actions of EGF (Sefton & Hunter, 1984; Hunter & Cooper, 1985). To date, only one protein besides the insulin receptor has been shown to be phosphorylated on tyrosine in response to insulin in intact cells (White *et al.*, 1985). Thus a search for common cellular substrates of the EGF- and insulin-receptor kinases may not be very fruitful.

Two other approaches are to look for possible common effects of EGF and insulin on enzymes such as cyclic AMP phosphodiesterase, adenylate cyclase, phosphoprotein phosphatases and protein kinases, and for possible common alterations in the phosphorylation states of glycogen synthase, acetyl-CoA carboxylase, ATP citrate lyase, pyruvate dehydrogenase, ribosomal protein S6 and other proteins.

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