Epidermal growth factor, like glucagon, exerts a short-term stimulation of alanine transport in rat hepatocytes

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INTRODUCTION

Epidermal growth factor (EGF) binds to specific receptors in liver plasma-membrane vesicles (O'Keefe et al., 1974) and isolated hepatocytes (Fehlmann et al., 1981). In combination with insulin and glucagon, EGF stimulates DNA synthesis and cell proliferation in hepatocytes in culture, and has been implicated in the hormonal control of liver regeneration (for a review see Leffert et al., 1979).

EGF has been shown to produce a variety of other effects on isolated hepatocytes. EGF mimics the effects of insulin in stimulating fatty acid synthesis and the phosphorylation of acetyl-CoA carboxylase and ATP citrate lyase (Holland & Hardie, 1985). Other insulin-like effects include stimulation of glycogen synthesis and inhibition of both the glucagon-induced increase in intracellular cyclic AMP and the stimulation of glycogen phosphorylase (Bosch et al., 1986).

Other effects of EGF differ from those of insulin. EGF causes a transient increase in cytosolic Ca\(^{2+}\) (Johnson et al., 1986; Bosch et al., 1986). EGF did not cause long-term induction of amino acid transport in primary cultures of hepatocytes but it inhibited that caused by glucagon and catecholamines, while not affecting that by insulin (Morin et al., 1981; Auburger et al., 1983; Visciano & Fehlmann, 1984). It is not at present clear which, if any, of the above effects are related to the initiation of cell proliferation by EGF. EGF has also been shown to stimulate both Na\(^+\) influx and Na\(^+\)/K\(^+\) ATPase activity in isolated hepatocytes (Fehlmann et al., 1981).

Na\(^+\)-dependent alanine transport into isolated hepatocytes is an electrogenic process which has been fully characterized (for a review see Kilberg et al., 1985). The initial rate of alanine transport is stimulated by glucagon by a protein-synthesis-independent mechanism (Edmondson & Lumeng, 1980). This effect of glucagon has been studied in detail (Moule et al., 1987), and it has been concluded that the primary effect of glucagon in this system is to stimulate the electroneutral influx of Na\(^+\) ions, thus increasing intracellular Na\(^+\) concentrations. This in turn increases electrogenic Na\(^+\)/K\(^+\) ATPase activity and causes membrane hyperpolarization. Since EGF exerts effects on Na\(^+\) influx and Na\(^+\)/K\(^+\) ATPase activity in hepatocytes (Fehlmann et al., 1981), which are similar to those of glucagon (Ihlenfeldt, 1981; Moule et al., 1987), it was decided to determine whether EGF also exerted a short-term stimulation of Na\(^+\)-dependent amino acid transport.

EXPERIMENTAL

Materials

EGF from mouse submaxillary glands either was obtained from Collaborative Research, Lexington, MA, U.S.A., or was Sigma Receptor Grade (Sigma Chemical Co., Poole, Dorset, U.K.). Identical results were obtained with EGF from both sources. Collagenase for the isolation of hepatocytes was purchased from Lorne Diagnostics, Bury St. Edmunds, Suffolk, U.K. Radioisotopes were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. All other reagents were of analytical grade.

Methods

Hepatocytes were isolated from 24 h-starved male Wistar rats as described previously (Bradford et al., 1985). Cells were incubated at a concentration of 8–12 mg of protein/ml in Krebs–Henseleit (1932) bicarbonate buffer containing 2% (w/v) dialysed bovine albumin (Fraction V) plus 20 mM-Mops at pH 7.4 under an atmosphere of O\(_2\)/CO\(_2\) (19:1) at 37 °C. In all experiments 1 mM-amino-oxycetate was present to inhibit completely the metabolism of alanine (Edmondson et al., 1977; Joseph et al., 1978). In experiments where the initial rate of transport was measured, transport was initiated by the addition to the cells of \[^{3}H\]alanine (final concn. 0.5 mM) plus \[^{14}C\]sucrose as a marker of the extracellular space. Transport was terminated after 2 min by centrifugal filtration of the cells through silicone oil into HClO\(_4\) as described previously (Joseph et al., 1978). Radioactivity in the pellet and supernatant was determined by liquid-scintillation counting. The amount of alanine in the pellet was corrected for alanine carried down in the extracellular water. Cell protein was measured by a biuret method (Gornall et al., 1949), with bovine albumin as a standard.

Abbreviations used: EGF, epidermal growth factor; Na\(^+\)/K\(^+\) ATPase, Na\(^+\)/K\(^+\)-transporting ATPase (EC 3.6.1.37).
Table 1. Effect of EGF on the transport of alanine in hepatocytes

Hepatocytes were preincubated as described in the Experimental section with or without 10 nM-EGF for 20 min. Transport was initiated by the addition of 0.5 mM-alanine, and the uptake of alanine was measured at the times shown. The values given are means ± S.E.M. from determinations on three separate cell preparations. Significance was assessed by a paired t test: *P < 0.05, **P < 0.01, ***P < 0.001 versus control.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control</th>
<th>+ EGF</th>
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<tr>
<td></td>
<td>(nmol/mg of protein)</td>
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<tr>
<td>1</td>
<td>0.83 ± 0.02</td>
<td>1.04 ± 0.04***</td>
</tr>
<tr>
<td>2</td>
<td>1.77 ± 0.08</td>
<td>2.02 ± 0.07***</td>
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<tr>
<td>3</td>
<td>2.67 ± 0.12</td>
<td>3.02 ± 0.12***</td>
</tr>
<tr>
<td>5</td>
<td>4.45 ± 0.18</td>
<td>4.97 ± 0.15***</td>
</tr>
<tr>
<td>10</td>
<td>8.02 ± 0.24</td>
<td>8.95 ± 0.25*</td>
</tr>
<tr>
<td>15</td>
<td>9.41 ± 0.23</td>
<td>10.5 ± 0.23**</td>
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RESULTS

Table 1 shows the effect of preincubation of hepatocytes with EGF on the time course of alanine uptake at 0.5 mM-alanine. Preincubation with 10 nM-EGF for 20 min stimulated the rate of transport in the initial linear phase by 15–25%; the stimulation was significant at each time point.

The concentration-dependence of the stimulation of alanine transport by EGF is shown in Fig. 1. A half-maximum effect was obtained at 1 nM-EGF, and the effect was maximal at 0.1 μM. This dose-dependence is very similar to that for the stimulation of Na⁺ and Rb⁺ transport by EGF in hepatocytes (Fehlmann et al., 1981). Other established effects of EGF on hepatocytes exhibit different dose-dependencies. Inhibition by EGF of the induction of α-aminoisobutyrate transport by glucagon in cultured hepatocytes was half-maximal at between 0.01 nM and 0.1 nM (Morin et al., 1981). Stimulation of glycogen synthetase activity by EGF in hepatocyte suspensions was half-maximal at 10 nM (Bosch et al., 1986). It has been shown that there are two different types of EGF receptors in hepatocytes, with K₀ values of 21 pM and 0.62 nM (Gladhaug & Christoffersen, 1987). The effects described here may therefore be mediated via the low-affinity receptor.

The stimulation of alanine transport by EGF was transient (Fig. 2). A maximum effect of approx. 30% stimulation of the initial rate of transport was obtained after 30 min, but the rates returned to control values after 90 min. Initial rates of transport did not alter significantly during incubation of hepatocytes for 90 min in the absence of EGF. Morin et al. (1981) and Auburger et al. (1983) have shown previously that 1 nM-EGF did not stimulate α-aminoisobutyrate transport when transport was measured after exposure of cultured hepatocytes to EGF for 3 h.

DISCUSSION

The results presented in this paper demonstrate that EGF exerts a transient stimulation of Na⁺-dependent alanine transport in hepatocytes. The time course of the stimulation of transport over the first 60 min is very similar to that observed with glucagon (Edmondson & Lumeng, 1980) and with dibutyryl cyclic AMP (Moule et al., 1987). The stimulation of transport observed with glucagon is biphasic; the second protein-synthesis-dependent phase of stimulation does not, however, occur with EGF. The magnitude of the stimulations obtained with glucagon and with EGF are similar. Preliminary results (not shown) indicate that the effect of EGF on transport, like that of glucagon, is abolished by ouabain.

A close correlation between cell membrane potential and the initial rate of Na⁺-dependent alanine transport in hepatocytes has been demonstrated, and glucagon activates transport by causing membrane hyperpolarization. This effect has been attributed to a primary increase in the rate of electroneutral Na⁺ influx, which stimulates electrogenic Na⁺/K⁺ ATPase activity (Moule

Fig. 1. Concentration-dependence for stimulation of alanine transport by EGF

Cells were preincubated with the concentrations of EGF shown for 30 min before the addition of 0.5 mM-alanine. Alanine uptake was determined after a further 2 min. The results shown are means ± S.E.M. of values obtained from three separate cell preparations. The rate of alanine uptake in the absence of EGF was 0.81 ± 0.02 nmol/min per mg of protein.
Epidermal growth factor and alanine transport

Fig. 2. Time course of the effect of EGF on the initial rate of alanine transport

Hepatocytes were incubated as described in the Experimental section and the time course was started by the addition of 10 mM-EGF. At the times indicated, the initial rate of alanine transport was measured as described in the legend to Fig. 1. Parallel experiments were performed in the absence of EGF; the initial rate of transport (0.84 ± 0.04 nmol/min per mg of protein) was found to be constant on preincubation of cells without EGF for periods up to 90 min. The results shown are means ± S.E.M. from eight separate cell preparations.

REFERENCES

et al., 1987). EGF has been shown to stimulate Na⁺/H⁺ exchange in a number of cell types (see Moolenaar et al., 1986). EGF and glucagon exert similar effects on Na⁺ uptake, Na⁺/K⁺ ATPase activity and Na⁺-dependent alanine transport in hepatocytes. An interesting possibility is that both EGF and glucagon stimulate a Na⁺/H⁺ exchange system in hepatocytes, thus indirectly causing membrane hyperpolarization. A similar mechanism has been proposed to explain stimulation of leucine transport by fetal-calf serum in Chang liver cells (Mitsumoto & Mohri, 1986).

At low concentrations of alanine, alanine transport in hepatocytes is a rate-determining step in alanine metabolism (Sips et al., 1980; McGivan et al., 1981). One metabolic function of the short-term stimulation of alanine transport by glucagon is to stimulate gluco-neogenesis in response to decreased blood glucose. It is unlikely, however, that such metabolic effects are important in EGF action, and the stimulation of alanine transport reported here is probably mainly a reflection of membrane hyperpolarization.

EGF has been shown to cause cell-membrane hyperpolarization in cultured pig thyroid cells (Bourke et al., 1986). Hyperpolarization of the cell membrane has also been observed in regenerating perfused rat liver. Cell hyperpolarization resulting from activation of Na⁺/K⁺ ATPase activity has been suggested to be an early event in rat liver regeneration (Wondergem & Harder, 1980). It is therefore possible that cell hyperpolarization by EGF may contribute to its eventual effect on hepatocyte proliferation.

This work was supported by the Medical Research Council.

Received 7 July 1987; accepted 29 July 1987