EGF INHIBITS GLUCAGON STIMULATION OF AMINO ACID TRANSPORT IN PRIMARY CULTURES OF ADULT RAT HEPATOCYTES

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1. Introduction

Epidermal growth factor (EGF) binds to specific plasma membrane receptors and stimulates growth and proliferation in a wide variety of cells in culture (review [1]). There is increasing evidence to suggest that the liver is an important target tissue for EGF action. Thus, specific binding sites for EGF have been characterized in liver plasma membranes [2] and isolated hepatocytes (M. F. et al., submitted). Furthermore, EGF is biologically active in hepatocytes. Indeed, a growth promoting effect of EGF has been demonstrated in neonatal [3] and adult [4,5] rat hepatocytes and intraperitoneal infusion of EGF has been reported to initiate hepatic DNA synthesis in vivo [6]. The effect of EGF on the initiation of DNA synthesis in liver cells was greatly amplified by the simultaneous presence of insulin and glucagon [3–6].

Here, we have investigated the effects of EGF, insulin and glucagon used individually or in combination, on amino acid transport in monolayer cultures of adult rat hepatocytes. While insulin and glucagon displayed additive stimulatory effects, EGF by itself had no significant effect on amino acid transport. The unexpected and intriguing finding was that EGF strongly inhibited the glucagon stimulation of amino acid transport but failed to affect the insulin stimulatory effect.

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2. Materials and methods

Hepatocytes, isolated from adult male Wistar rats as in [7] were allowed to plate on collagen-precoated culture dishes for 4 h at 37°C in Waymouth's medium in the presence of 10% fetal calf serum [8]. After the attachment period, the medium was replaced with Waymouth's medium, without serum, containing 0.2% defatted bovine serum albumin (BSA). Monolayer cultures were incubated at 37°C for an additional 20 h. Antibiotics (penicillin, 100 units/ml and streptomycin, 100 μg/ml) were continuously present in Waymouth's media.

For transport studies, cells were washed 3 times with 2 ml Krebs-Ringer bicarbonate (KRB) buffer and incubated in 0.9 ml of KRB buffer containing 1% defatted BSA, bacitracin (0.8 mg/ml) and gentamycin (50 μg/ml), in the absence or presence of hormones for 3 h at 37°C. At the end of the incubation period, transport assays were initiated by adding 0.1 ml of KRB buffer containing α-amino[^{14}C]isobutyrate ([^{14}C]AIB) (0.2 μCi, 0.1 mM final conc.) and [^{3}H]-inulin (1 μCi). After 15 min the reaction was terminated by aspirating the radioactive medium and by washing the cells 3 times with 2 ml chilled KRB buffer. The cells were digested with 0.5 ml 0.2 N NaOH and counted for ^{14}C and ^{3}H. The transport data were corrected for extracellular trapping, and normalized per mg protein.

Porcine monocomponent insulin and highly purified porcine glucagon were gifts from Novo; epidermal growth factor was purchased from Collaborative Res.

3. Results

The effects of insulin, glucagon, dibutyryl cyclic AMP (Bt_2cAMP), and EGF on AIB transport were

Table 1
Effects of insulin, glucagon, Bt_2cAMP and EGF on α-aminoisobutyrate (AIB) transport in primary cultures of rat hepatocytes

		AIB influx (nmol . mg protein^{-1} . 15 min^{-1})	% Increase above basal
Basal	(n = 8)	0.67 ± 0.11	–
Insulin	(n = 5)	2.41 ± 0.21	260
Glucagon	(n = 8)	3.60 ± 0.48	437
Bt_2cAMP	(n = 5)	360 ± 0.72	437
EGF	(n = 8)	0.78 ± 0.28	16
Insulin + EGF	(n = 5)	2.82 ± 0.39	321
Glucagon + EGF	(n = 8)	1.45 ± 0.18	116
Bt_2cAMP + EGF	(n = 5)	1.53 ± 0.21	128
Insulin + glucagon	(n = 5)	4.71 ± 0.39	603
Insulin + glucagon + EGF	(n = 5)	2.78 ± 0.54	314

Monolayers of rat hepatocytes were incubated for 3 h at 37°C without (basal) or with 10 nM insulin, 100 nM glucagon, 10 μM Bt_2cAMP or 1 nM EGF, used alone or in combination. The influx of 0.1 mM [^{14}C]AIB was then measured over a 15 min period. Each value represents the mean ± SE of n separate expt.

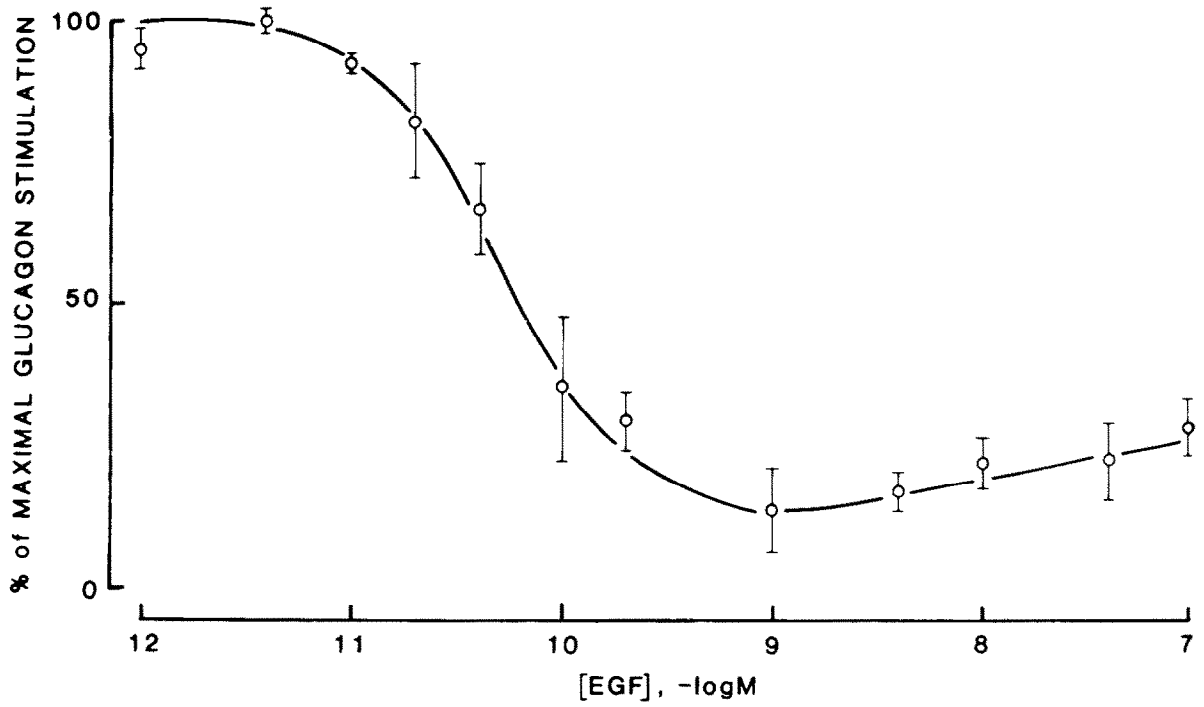

Fig.1. Dose dependence of the effect of EGF on the stimulatory effect of glucagon on α-aminoisobutyrate transport. Monolayers were incubated for 3 h at 37°C with varying concentrations of EGF (0.01–100 nM) in the presence of 100 nM glucagon. The uptake of 0.1 mM α-amino[^{14}C]isobutyrate was then measured over a 15 min period. Results are expressed as the percentage of maximal glucagon stimulation. Each point represent the mean ± SE of 3 separate expt.

investigated in primary cultures of rat hepatocytes after a 3 h exposure to each agent used alone or in combination. As reported, insulin [8,9] and glucagon [9] were found to stimulate AIB transport in primary cultures of hepatocytes by 260% and 440%, respectively (table 1). EGF (1 nM) had no significant effect on AIB transport. Raising the concentration of EGF to 1 μM did not affect AIB transport, either in monolayer cultures or in fresh suspensions of rat hepatocytes (not shown). However, EGF strongly inhibited the glucagon-induced stimulation of AIB transport, and was without effect on the stimulation of amino acid transport by insulin (table 1). Consistent with this finding, we observed that EGF also inhibited the effect of Bt_2cAMP.

As observed in freshly isolated hepatocytes [7], insulin and glucagon exerted additive effect on AIB transport in primary cultured hepatocytes (table 1). Interestingly, the inhibitory effect of EGF on the glucagon-stimulated AIB transport persisted in the presence of insulin (table 1).

The dose-dependence of the inhibitory effect of EGF on the glucagon stimulation of AIB transport is shown in fig.1. The inhibitory effect of EGF could be detected at as low as 0.01 nM peptide. The half-maximal inhibition occurred with EGF at 0.067 ± 0.018 nM (n = 3), and maximal effect (~85% inhibition) was observed with EGF at 1 nM. With greater concentrations of EGF (4–100 nM), this inhibition was slightly reduced (fig.1).

4. Discussion

We have shown here that EGF selectively inhibits the glucagon stimulation of amino acid transport in primary cultured rat hepatocytes. The observation that EGF inhibits the cAMP stimulatory effect as well as the glucagon effect, along with the fact that EGF does not inhibit glucagon binding in hepatocytes [10], indicate that the inhibitory effect of EGF occurs at a post-receptor step of glucagon action. Glucagon stimulated amino acid transport in freshly isolated hepatocytes by inducing the synthesis of high affinity carrier proteins which are not expressed under basal conditions [7]. In [9] glucagon stimulated amino acid transport in monolayer cultures of rat hepatocytes and this was also found to result from the synthesis of high affinity transport proteins (O. M., unpublished). Many intracellular events are likely to occur between the transient raise in intracellular cAMP induced by glucagon and the synthesis of specific proteins. The identification of the step(s) affected by EGF could shed some light on the mechanism of action of this peptide on the control of protein synthesis.

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