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EFFECT OF EPIDERMAL GROWTH FACTOR ON CHOLECYSTOKININ-INDUCED AMYLASE RELEASE AND INOSITOL 1,4,5-TRISPHOSPHATE PRODUCTION IN PANCREATIC ACINAR CELLS

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SUMMARY: In the present study we investigated the role of epidermal growth factor (EGF) in cholecystokinin-octapeptid (CCK8)-induced inositol 1,4,5-trisphosphate (IP_3) production and amylase release. The data show that high EGF concentrations (90 nM) increased the basal amylase release, but did not increase the acinar IP_3 content. High EGF concentrations shifted the dose-response curve for CCK8-induced amylase release to lower CCK8 concentrations, whereas low EGF concentrations (17 nM) shifted this dose-response curve to higher CCK8 concentrations. 17 nM EGF inhibited CCK8-induced IP_3 production during the whole period of observation of 5 min, whereas 90 nM EGF inhibited only the initial component (15 s) of CCK8-induced IP_3 production. At later time points, 90 nM EGF increased CCK8-induced IP_3 production. We conclude that low concentrations of EGF are inhibitory on CCK8-induced IP_3 production and amylase release, whereas high EGF concentrations stimulate CCK8-induced amylase release.

The stimulation of phosphatidylinositol 4,5-bisphosphate hydrolysis by a wide array of hormones, neurotransmitters, and growth factors to yield the second messengers inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol is well documented [1,2]. However, despite the considerable effort invested in dissecting the molecular events that underlie receptor modulation of PLC activity, the mechanism by which binding of ligands to their receptors triggers activation of phosphoinositide-specific phospholipase C (PLC) is not entirely clear.

In pancreatic acinar cells, growth factors such as basic fibroblast growth factor (bFGF) induce IP_3 accumulation, release of intracellular calcium and amylase release [3]. Epidermal growth factor (EGF) induces release of intracellular calcium, whereas amylase release and the IP_3 level in the acinar cells were not significantly altered [3]. Previously it has been shown that low concentrations of EGF inhibit CCK8-induced IP_3-production and release of intracellular calcium [4]. However, the effect of EGF on CCK8-induced amylase release remained unclear.

In this study we therefore investigated the effect of different EGF concentrations on cholecystokinin-octapeptide (CCK8)-induced IP_3-production and amylase release in rat pancreatic acini.

MATERIALS AND METHODS

Materials:

Cholecystokinin-octapeptide, epidermal growth factor, collagenase type III, and soy bean trypsin inhibitor were from Sigma (München, FRG). Amylase assay kit was from Boehringer

0006-291X/93 $4.00

(Mannheim, FRG). The [^{3}H]IP_3 radioreceptor assay was purchased from New England Nuclear (NEN, Dreieich, FRG).

Methods:

Preparation of isolated rat pancreatic acinar cells: Acinar cells were isolated from the rat pancreas by collagenase digestion as described [5,6]. The acini were suspended in Na^+-Krebs Ringer Hepes (KRH) buffer of the following composition (in mM): 145 NaCl; 4.7 KCl; 2 $MgCl_2$; 1.2 KH_2PO_4; 2 $CaCl_2$; 10 glucose; 0.2% bovine serum albumin; 0.01% soybean trypsin inhibitor; 10 Hepes/NaOH pH 7.4.

Measurement of amylase release: For measurement of amylase release aliquots of five ml were incubated with the appropriate secretagogues for 30 min at 37°C under continuous supply of 100% O_2. 300 µl aliquots were removed at the beginning and at the end of the incubation and were immediately centrifuged at 1000 x *g* for one minute. Each sample was performed in duplicate. 200 µl supernatant were removed and assayed for amylase activity by detecting the hydrolysis rate of p-nitrophenyl-α,D-maltoheptaoside with the Boehringer assay kit. Amylase release was expressed as percent of the total content of amylase at the beginning of the incubation.

Mass measurement of IP_3 production in the acini: IP_3 production in the acini was measured by displacement of bound [^{3}H]IP_3 using a IP_3-specific radioreceptor assay. Briefly, acini from two rats were suspended in 30 ml Na^+-based Hepes-buffered Ringer solution. Aliquots of two ml were incubated in a continuously stirred cuvette at 37°C. After the indicated time, 200 µl aliquots were removed and mixed with an equal volume ice-cold trichloroacetic acid (20%) and stored at 4°C for one h. After centrifugation for 15 min at 5000 x *g* in a cold Eppendorf centrifuge, 300 µl of the supernatants were extracted with 800 µl for 1,1,2-trichloro-1,2,2-trifluoroethan/trioctylamine (3:1, v/v). The IP_3 content of the upper aquous phase was determined in duplicate using the radioreceptor assay for IP_3 and was performed according to the assay protocol.

Protein was measured according to the method of Bradford [7].

Statistical analysis: The data presented are means ± standard error of the mean (SEM) of three or more experiments. The statistical significance was calculated using Student's t-test for paired values.

RESULTS

Effect of EGF on amylase release from isolated rat pancreatic acinar cells.

Basal amylase secretion was approximately 5% of total amylase content present at the beginning of the incubation. As shown in Fig. 1, EGF at a concentration of 17 nM did not cause a

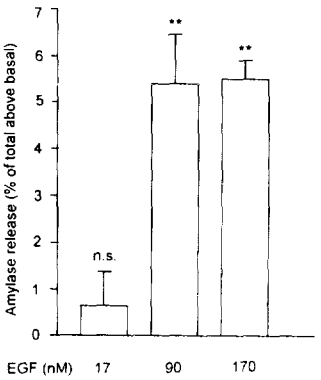

Figure 1. Effect of EGF on amylase secretion.

Rat pancreatic acini were incubated for 30 min with various concentrations of EGF and the amount of amylase secreted into the medium was quantitated. The results are quantitated as % of total amyase content present at the beginning of the incubation. Results are mean values ± SEM of four to eight different experiments. *, $P<0.05$; **, $P<0.01$.

significant increase of amylase release from the acini above basal values. However, rather high EGF concentrations of 90 or 170 nM caused an amylase release of 5.4±1.0 and 5.5±0.4% of total above basal, respectively (n=6, P<0.01).

Effect of EGF on CCK8-induced amylase release.

The dose-response curve for CCK8-stimulated amylase release is biphasic, showing increase at low and decrease of the maximum stimulated amylase release at supramaximal CCK8 concentrations (>1 nM) (Fig. 2A). Maximum CCK8-stimulated amylase release of 11.0±1.4% of total above basal was observed at 1 nM CCK8 (n=6). Low concentrations of EGF (17 nM), which had no effect on the basal amylase release, shifted the upstroke of the dose-response curve for CCK8-stimulated amylase release to higher CCK8 concentrations. Half maximal stimulation was observed at 40 pM in the absence and 0.2 nM in the presence of EGF (n=4). At the supramaximal CCK8 concentration of 100 nM, EGF increased amylase release from 5.1±1.2 to 8.4±1.0% of total above basal (n=7, P<0.01).

Different from the effect of low EGF concentrations on CCK8-induced amylase secretion, high EGF concentrations (90 nM) shifted the dose-response curve for CCK8-stimulated amylase release to lower CCK8 concentrations. Half maximal stimulation occurred at 50 pM in the absence and 15 nM in the presence of EGF (Fig. 2B, n=4).

Effect of EGF on CCK8-induced IP_3 production.

According to the inhibitory role of EGF in CCK8-induced amylase release, low EGF concentrations (17 nM) inhibited IP_3-production in response to CCK8 (100 nM) (Fig. 3). This

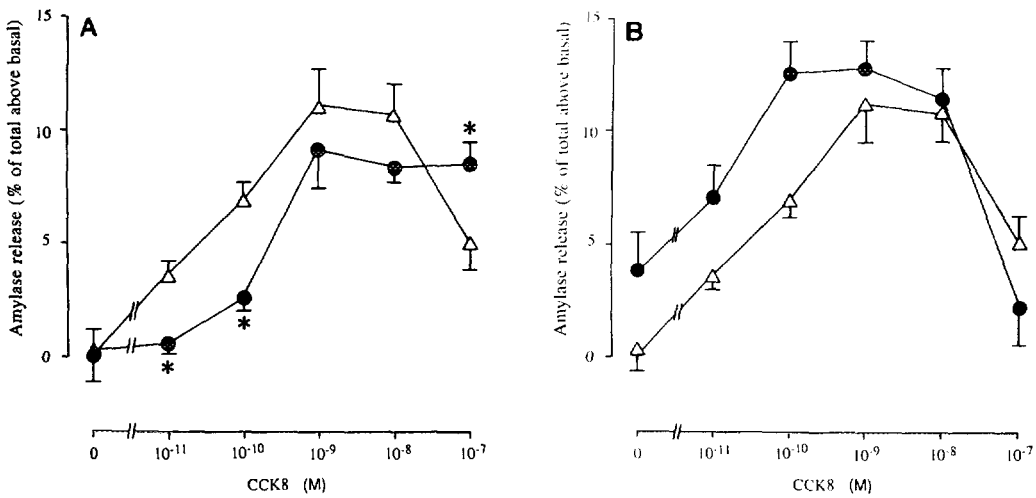

Figure 2. Effect of EGF on CCK8-induced amylase secretion.

Acini were incubated with various concentrations of CCK8 either in the presence (●—●) or absence (△—△) of EGF (Fig. 2A: 17 nM, Fig. 2B: 90 nM). Results are mean values ± SEM of four different experiments. Asterisks (*) indicate significant differences (P<0.05) as compared to the value in the absence of EGF.

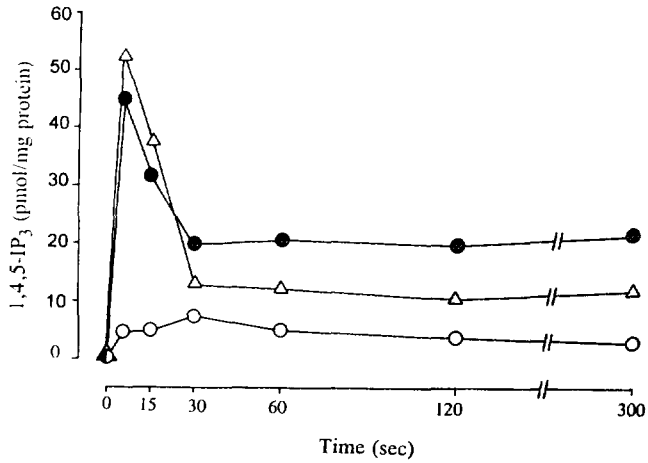

Figure 3. Time course for the CCK8-induced IP_3 production in the presence and absence of EGF.

Acini were incubated with CCK8 (100 nM) without (△—△) and with 17 nM EGF (○—○) or 90 nM EGF (●—●) as described in materials and methods. The experiment shown is representative for four different experiments.

inhibitory effect was observed during the complete time course of five minutes, i.e. inhibition of the initial peak IP_3-production as well as the plateau phase.

High concentrations of EGF (90 nM) inhibited CCK8-induced peak IP_3-production during the first fifteen seconds after beginning of the incubation. However, at later time points 90 nM EGF showed an increase of CCK8-induced plateau IP_3-production.

DISCUSSION

EGF stimulates basal and CCK8-induced enzyme secretion in pancreatic acinar cells which had been preincubated with the growth factor in short term cell culture [8]. Acute effects of EGF include stimulation phosphoinositide hydrolysis in several transformed cells that overexpress EGF receptors [9-13]. In contrast it has been found that this growth factor has little effect on this process in most non-transformed cells [14-17]. Similar to a recent study [3] we have shown that high concentrations of EGF increase the intracellular calcium concentration in fura-2-loaded pancreatic acini, but failed to induce substantial IP_3- or cAMP-production (data not shown).

In the present study high EGF concentrations induced a release of amylase in pancreatic acinar cells (Fig. 1). However, incubation of pancreatic acini with both low EGF concentrations and CCK8 showed that EGF inhibits CCK8-induced IP_3-production (Fig. 3), the release of calcium from intracellular stores and CCK8-induced Cl^- conductance in zymogen granules [4,18]. In agreement with these data, low concentrations of EGF inhibited the upstroke of the dose response curve for CCK8-stimulated amylase release (Fig. 2A). EGF receptors functionally interact with α-subunits of G_i-type G-proteins in rat pancreatic acini [19]. The inhibition of PLC acitivity in CCK-stimulated pancreatic acinar cells by EGF might be mediated by these G_i-proteins.

The initial peak increase of IP_3 in response to calcium-mobilizing secretagogues initiates rather than causes substantial amylase release. Inositol 1,3,4,5-tetrakisphosphate formed by

phosphorylation of IP_3, together with IP_3 itself mediate the entry of calcium from the extracellular compartment [20]. Sustained increase in calcium depends on the presence of extracellular calcium, reflects increased calcium entry across the plasma membrane and probably mediates sustained amylase release [21]. Therefore, the increase of the acinar IP_3-production during the plateau phase of CCK8-induced IP_3-production could explain the increase of amylase release in response to both high EGF concentrations plus CCK8 as compared to CCK8 alone (Fig. 2B) despite the inhibitory effect of high EGF concentrations during the initial peak of CCK8-induced IP_3-production.

The downstroke of the dose response curve for CCK8-stimulated enzyme secretion is correlated with further increase of CCK8-induced IP_3- and diacylglycerol-production and mobilization of calcium from intracellular stores [22,23]. As discussed elsewhere [6] it had been suggested that overstimulation of PLC mediates the decline of the dose response curves for CCK8-stimulated enzyme secretion. If this hypothesis is correct, the decrease of supramaximal activation of PLC at high CCK8 concentrations by low EGF concentrations should increase enzyme secretion. Indeed, low EGF concentrations prevented the downstroke of the dose response curves for both CCK8-stimulated amylase release (Fig. 2A).

We conclude that EGF has dual effects on CCK8-induced IP_3-production and amylase secretion: inhibition at lower EGF concentrations and stimulation at higher EGF concentrations.

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