Vol. 177, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1991 Pages 166-170

BASIC FIBROBLAST GROWTH FACTOR IS A CALCIUM-MOBILIZING SECRETAGOGUE IN RAT PANCREATIC ACINI

Bysani Chandrasekar and Murray Korc

Departments of Medicine and Biological Chemistry, University of California, Irvine, CA 92717

Received April 8, 1991

Basic fibroblast growth factor (bFGF) induced a marked increase in the levels of inositol 1,4,5-trisphosphate [Ins(1,4,5)P3] and a rapid rise in cytosolic free calcium $[Ca^{2+}]_i$ levels in rat pancreatic acini. The bFGF-mediated calcium transient was not dependent on the presence of extracellular calcium, and was abolished by pretreatment of acini with carbachol. bFGF stimulated amylase release in pancreatic acini in a monophasic, dose-dependent manner, and this effect was blocked by neutralizing anti-bFGF antibodies. At much higher concentrations, epidermal growth factor (EGF), but not insulin-like growth factor-I (IGF-I), partially mimicked some of the actions of bFGF. These findings suggest that bFGF is a previously unrecognized calcium-mobilizing pancreatic secretagogue that may participate in the regulation of pancreatic exocrine function.

bFGF is a mitogenic polypetide that regulates numerous biological functions in a variety of cells (1). Initiation of bFGF action is believed to be dependent on its binding to a specific cell-surface tyrosine kinase receptor (2). bFGF is capable of activating phospholipase C-γ (PLC-γ), an enzyme that induces the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2), thereby generating sn-1,2-diacylglycerol (DG) and inositol 1,4,5-trisphosphate [Ins(1,4,5)P3] (3). DG activates protein kinase C (PKC), whereas Ins(1,4,5)P3 releases calcium from intracellular stores (4-5). The ability of bFGF to initiate PIP2 hydrolysis suggests that this pathway may play a pivotal role in mediating the actions of bFGF in some cell types.

We have previously reported that bFGF inhibits EGF binding in pancreatic acini (6), and that inhibition of EGF binding in these cells by cholecystokinin-octapeptide (CCK8), bombesin, and carbachol is mediated via calcium (7). Therefore, in the present study, we sought to determine whether bFGF modulates cytosolic calcium levels in the pancreatic acinar cell, and whether it mimics the biological actions of other calcium-mobilizing agonists in this cell type.

MATERIALS AND METHODS

Cell Isolation. Pancreatic acini were prepared by collagenase digestion of pancreatic tissue from overnight-fasted male Sprague-Dawley rats, and incubated in HEPES-buffered Ringer's (HR)

0006-291X/91 $1.50

solution (8). Unless otherwise specified, cells were maintained at 37°C. Cell viability, monitored with trypan blue, was always greater than 95%. Data were analyzed by analysis of variance (ANOVA), using the StatView 512 computer program.

Measurement of Inositol 1,4,5-trisphosphate and Calcium Transients. Following incubation of acini with agonists, reactions were terminated with 11% (final concentration) trichloroacetic acid (9). The supernatants were extracted with 1,1,2-trichloro-1,2,2-trifluoro-ethane-trioctylamine and analyzed for Ins(1,4,5)P3 content by a competitive binding assay (9). Calcium transients were measured with a Hitachi dual-wavelength fluorometer, using Fura-2 as the calcium indicator (10).

Secretion Studies. Acini were incubated for 30 min with carbachol, recombinant human bFGF (California Biotechnology, Mountain View, CA), recombinant human EGF (Chiron, Inc., Emmeryville, CA), recombinant human IGF-1 (AMGen Biologicals, Inc., Thousand Oaks, CA), and neutralizing anti-bFGF antibodies (Sigma Co., St. Louis, MO). Amylase release into the medium was measured by the Phadebas blue starch method, and expressed as units of activity per mg of acinar cell protein, based on the μmoles of maltose released/min (6, 8).

RESULTS

bFGF Actions on Ins(1,4,5)P3 and Calcium Transients. bFGF increased Ins(1,4,5)P3 levels within 5 sec, one-half maximal and maximal stimulation occurring at 10 pM and 50 pM, respectively (Table 1). Carbachol (30 μM) increased Ins(1,4,5)P3 levels to a greater extent than bFGF, EGF (16.5 nM) was only minimally effective ($p>0.05$), and IGF-I (16.5 nM) was without effect (Table 1). The actions of carbachol, but not bFGF, were blocked by atropine (Table 1). In the case of both agonists, Ins(1,4,5)P3 levels declined after 30 sec (data not shown).

bFGF induced a rapid and dose-dependent increase in $[Ca^{2+}]_i$ levels, threshold and maximal effects occurring at 0.5 pM and 5 pM, respectively (Fig. 1). At maximally effective concentrations, bFGF (5 pM) and carbachol (30 μM) increased $[Ca^{2+}]_i$ levels by 255±11% (mean±SE, n=4) and 351±11% (mean±SE, n=3) above control values, respectively. Thus,

TABLE 1

Effects of bFGF and other agonists on Ins(1,4,5)P3 levels

Addition	Ins(1,4,5)P3 (pmol/mg protein)	Addition	Ins(1,4,5)P3 (pmol/mg protein)
None	2.43±0.33	EGF (16.5 nM)	4.00±0.18
bFGF (0.5 pM)	4.41±0.54	IGF-1 (16.5 nM)	2.32±0.12
bFGF (5 pM)	7.01±0.24*	Carbachol (30 μM)	36.66±1.48**†
bFGF (30 pM)	13.97±1.31**	Carbachol (30 μM)	2.80±0.31
bFGF (50 pM)	18.10±0.86**	+ atropine (1 mM)	
bFGF (0.5 nM)	18.74±0.72**	Carbachol (30 μM)	37.89±1.07**†
bFGF (50 pM)	18.40±0.43**	+ bFGF (50 pM)	
+ atropine (1 mM)			

Pancreatic acini were incubated for 5 sec in the absence or presence of the indicated additions. Values are the means±SE of 3 experiments. *$p<0.0001$, **$p<0.00001$, when compared with control value; †$p<0.0001$, when compared with 50 pM bFGF.

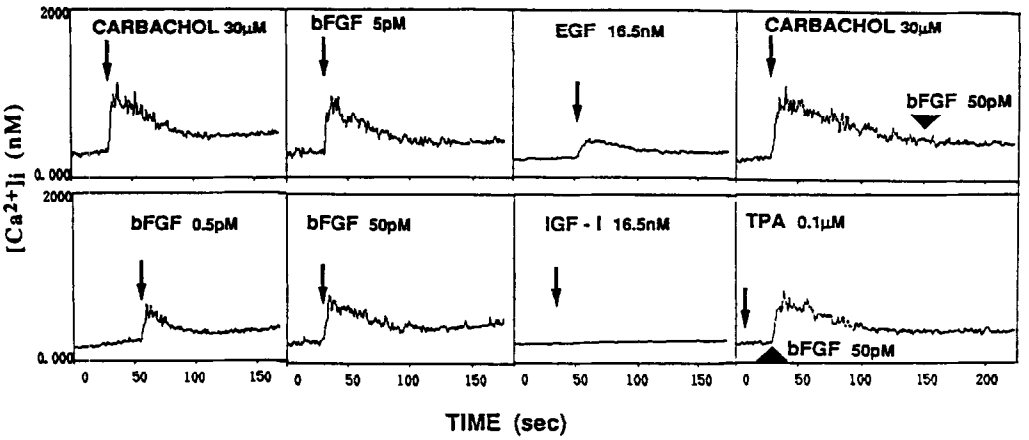

Fig. 1. Effects of growth factors and carbachol on cytosolic calcium levels. Pancreatic acini were maintained at 23°C, then transferred to 37°C prior to making the indicated additions. Arrows denote the primary additions. Arrowheads denote the secondary additions, which were made following the primary additions. Tracings shown are representative of 3 to 4 experiments.

carbachol exerted a greater effect on $[Ca^{2+}]_i$ than bFGF ($p<0.01$). The bFGF-induced calcium transients were not altered by adding 0.1 mM EGTA to calcium-deficient HR buffer (data not shown), or by preincubation with 0.1 μM tetradecanoyl-phorbol acetate (TPA; Fig. 1). However, the calcium transient was abolished by preincubating acini with 30 μM carbachol. EGF (16.5 nM) produced a small calcium transient, whereas IGF-I (16.5 nM) was without effect (Fig.1).

bFGF stimulated amylase release in pancreatic acini in a dose-dependent manner (Table 2). One-half maximal and maximal stimulation of amylase release occurred at 10 pM and 50 pM bFGF, respectively. At concentrations of 50 to 500 pM, bFGF was 69% as effective as 30 μM

TABLE 2

Effects of bFGF and other agonists on amylase release

Addition	Amylase Release (Units/mg protein)	Addition	Amylase Release (Units/mg protein)
None	2.73±0.22	EGF (16.5 nM)	3.98±0.05
bFGF (0.5 pM)	4.48±0.47*	IGF-1 (16.5 nM)	3.34±0.31
bFGF (5 pM)	5.80±0.46*	Carbachol (30 μM)	14.59±0.58***†
bFGF (30 pM)	7.11±0.53**	Carbachol (30 μM)	3.13±0.20
bFGF (50 pM)	10.20±0.56***	+ atropine (1 mM)	
bFGF (0.5 nM)	10.87±0.42***	Carbachol (30 μM)	15.18±0.46***†
bFGF (5 nM)	11.49±0.26***	+ bFGF (50 pM)	
bFGF (50 pM)	3.37±0.26	bFGF (50 pM)	10.27±0.48***
+ anti-bFGF antibody		+ atropine (1 mM)	

Pancreatic acini were incubated for 30 min in the absence or presence of the indicated additions. Values are the means± SE of 3 experiments. *$p<0.001$, **$p<0.0001$, ***$p<0.00001$ when compared with control value; †$p<0.0001$, when compared with 50 pM bFGF.

carbachol. In contrast, EGF and IGF-I were without effect. The actions of 50 pM bFGF were completely blocked by a neutralizing monoclonal anti-bFGF antibody (0.9 μg/ml). In contrast, the actions of carbachol, but not bFGF, were blocked by atropine. Increasing the concentration of bFGF to 5 nM caused a slight but insignificant additional increase in amylase release (Table 2), whereas increasing the carbachol concentration to 0.3 mM caused a lesser stimulation of amylase release than that observed at 30 μM carbachol (data not shown).

DISCUSSION

In the present study we have determined that bFGF is a pancreatic digestive enzyme secretagogue. The magnitude of the bFGF-induced secretory response was 30% smaller than that observed with carbachol. However, on a molar basis, bFGF was more potent than carbachol. Thus, bFGF compares favorably with CCK_8, a physiological regulator of pancreatic exocrine function that exerts maximal effects on amylase release at concentrations of 0.1 to 0.3 nM (11).

In contrast to carbachol and CCK_8 (11), supramaximal concentrations of bFGF did not exert a lesser effect on amylase release than maximally effective concentrations. Nonetheless, three lines of evidence indicate that bFGF, like carbachol and CCK_8, is a calcium mobilizing agonist in the pancreatic acinar cell. First, bFGF rapidly increased the mass levels of Ins(1,4,5)P3, a compound that releases calcium from intracellular stores (5). Second, bFGF rapidly induced a calcium transient in pancreatic acini, and this effect did not require extracellular calcium. Third, the ability of bFGF to raise $[Ca^{2+}]_i$ levels was not altered by TPA, but was abolished by pretreatment of acini with carbachol, a G-protein coupled cholinergic agonist. Similar extinctions of calcium transients occur when G-protein coupled calcium-mobilizing agonists are added to acini sequentially, ostensibly as a result of the depletion of Ins(1,4,5)P3-sensitive calcium pools (11). Although bFGF is a tyrosine kinase coupled PLC-γ activator, these observations suggest that bFGF acts on the same intracellular calcium pools as carbachol, and that carbachol does not inhibit bFGF action as a result of PKC activation.

IGF-1 failed to generate Ins(1,4,5)P3, induce calcium transients, or stimulate amylase release. A superphysiological concentration of EGF produced a small calcium transient, and exerted statistically insignificant stimulatory effects on both Ins(1,4,5)P3 levels and amylase release. As in the case of bFGF, IGF-1 and EGF bind and activate cell-surface tyrosine kinase receptors. However, the bFGF receptor exhibits a tyrosine kinase domain that is separated into two contiguous regions and an extracellular domain that has immunoglobulin-like regions, the

IGF-1 receptor is heterotetrameric, and the EGF receptor is monomeric (12). Therefore, it is possible that the bFGF receptor has unique features that allow it to efficiently generate inositol phosphates and rapidly raise $[Ca^{2+}]_i$ levels in the pancreatic acinar cell. In conjunction with its ability to inhibit EGF binding (6) and stimulate amylase secretion, these observations suggest that bFGF may exert important regulatory roles in the pancreas in both normal and disease states.

ACKNOWLEDGMENTS

This study was supported by NIH Grants CA-40162 and DK-32561 to M.K.

REFERENCES

1. Burgess, W., and Maciag, T. (1989) Ann. Rev. Biochem. 58, 575-606.
2. Coughlin, S.R., Barr, P.J., Cousens, L.S., Fretto, L.J., and Williams, L.T. (1988) J. Biol. Chem. 263, 988-933.
3. Kuo, M.D., Huang, S.S., and Huang, J.S. (1990) J. Biol. Chem. 265, 16455-16463.
4. Nishizuka, Y. (1988) Nature 334, 661-665.
5. Berridge, M.J., and Irvine, R.F. (1989) Nature 341, 197-205.
6. Korc, M., Matrisian, L.M., Planck, S.R., and Magun, B.E. (1983) Biochem. Biophys. Res. Commun. 111, 1066-1073.
7. Korc, M., Matrisian, L.M., and Magun, B.E. (1984) Proc. Natl, Acad. Sc. (USA) 81, 461-465.
8. Williams, J.A., Korc, M., and Dormer, R.L. (1978) Am. J. Physiol. 235, E517-E524.
9. Bredt, D.S., Mourey, R.J. and Snyder, S.H. (1989) Biochem. Biophys. Res. Commun. 159, 976-982.
10. Stuenkel, E., Tsunoda, Y., and Williams, J.A. (1989) Biochem. Biophys. Res. Commun. 158, 863-869.
11. Gardner, J.D. and Jensen, R.T. (1986) Receptors and cell activation associated with pancreatic enzyme secretion. Ann. Rev. Physiol. 48, 103-117.
12. Yarden, Y. and Ullrich, A. (1988) Ann. Rev. Biochem. 57, 443-478.