

## DIRECT TROPHIC EFFECTS OF FIBROBLAST GROWTH FACTORS ON RAT PANCREATIC ACINAR CELLS IN VITRO

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We examined the effects of fibroblast growth factors (FGFs) on rat pancreatic acinar cells in primary culture. Both basic and acidic FGF stimulated [<sup>3</sup>H]thymidine incorporation in a dose-dependent fashion. Maximum effects of 6-7 fold over control were seen with 1 nM bFGF or 100 nM aFGF. These data indicate that FGFs are potent stimulants of rat pancreatic acinar cell DNA synthesis. Therefore, FGFs may play an important role in long term regulation of the exocrine pancreas in vivo. We also examined the interaction of bFGF with the pancreatic secretagogues CCK and carbachol. Effects of CCK, which is itself an acinar cell trophic factor, were additive with those of bFGF. In contrast, carbachol, which has no growth stimulatory effect, did not affect bFGF mediated stimulation of DNA synthesis. These data suggest that the mechanisms involved in acinar cell growth regulation are independent from those involved in secretion. © 1993 Academic Press, Inc.

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Fibroblast growth factor (FGF) was first isolated from bovine pituitary as a factor that stimulates the growth of BALB/c 3T3 cells (1). Since then it has been found that FGF exists in acidic and basic forms and that FGFs stimulate growth in a wide variety of cells and are involved in numerous biological processes including wound repair, embryo development, neuron survival, bone formation, and angiogenesis (for reviews see Refs. 1648,1669). Inappropriate expression of FGF receptors may lead to increased cell growth rates and may be involved in cell transformation (2,3).

FGFs act through specific cell surface receptors and activate second messengers in a cascade originating from their intrinsic tyrosine kinase activity (4,5). Recently it was reported that basic FGF (bFGF) stimulated amylase release from rat pancreatic acini in a dose dependent fashion and its effect was abolished with anti-bFGF antibodies (6). These findings indicate the presence of FGF receptors on rat pancreatic acinar cells. However, no physiologic role for FGF in the pancreas has been defined. In this study, we used a primary culture system of rat pancreatic acinar cells to examine the direct effects of FGFs on the growth of these cells.

## MATERIALS AND METHODS

### **Materials**

The following were purchased: Dulbecco's modified Eagle's medium, fetal bovine serum, penicillin, streptomycin, and amphotericin B from GIBCO (Grand Island, NY); Waymouth medium MB752/1, soybean trypsin inhibitor type I-S, isobutylmethylxanthine (IBMX), and bovine serum albumin (BSA) fraction V, from Sigma Chemical (St. Louis, MO); chromatographically purified collagenase from Worthington Biochemical (Freehold, NJ); rat tail collagen type I from Becton Dickinson Labware (Bedford, MA); CCK8 sulfated from Bachem (Torrance, CA); basic FGF (bFGF) and acidic FGF (aFGF) from UBI (Lake Placid, NY); [<sup>3</sup>H]thymidine from Amersham (Arlington Heights, IL); trichloroacetic acid from J.T.Baker (Phillipsburg, NJ)

### **Isolation and Culturing of Pancreatic Acini**

Isolated rat pancreatic acini were prepared by minor modification of previously published methods (7). The basic medium used for isolation was a Dulbecco's modified Eagle medium (DME) to which soybean trypsin inhibitor (0.1 mg/ml), penicillin (100 U/ml), streptomycin (100 µg/ml) and amphotericin B (50 µg/ml) were added. Acini prepared by collagenase digestion and mechanical shearing were purified by filtration through 150 µm nylon mesh and centrifuged through DME buffer containing 4% bovine serum albumin. The acini were then rinsed and resuspended at a density of 700-900 µg protein/ml in culture medium and aliquoted at 1 ml per well of precoated 24 well cell culture cluster. The plates were precoated with 150 µg of rat tail collagen per well and air dried over night. The culture medium consisted of Waymouths medium supplemented with soybean trypsin inhibitor (0.2 mg/ml), 2.5% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 µg/ml), amphotericin B (50 µg/ml) and IBMX (0.5 mM). After 1 hour preincubation, FGFs and/or CCK8 or carbachol were added to each well. Acini were maintained in a humidified atmosphere of 5% CO<sub>2</sub> in air at 37°C.

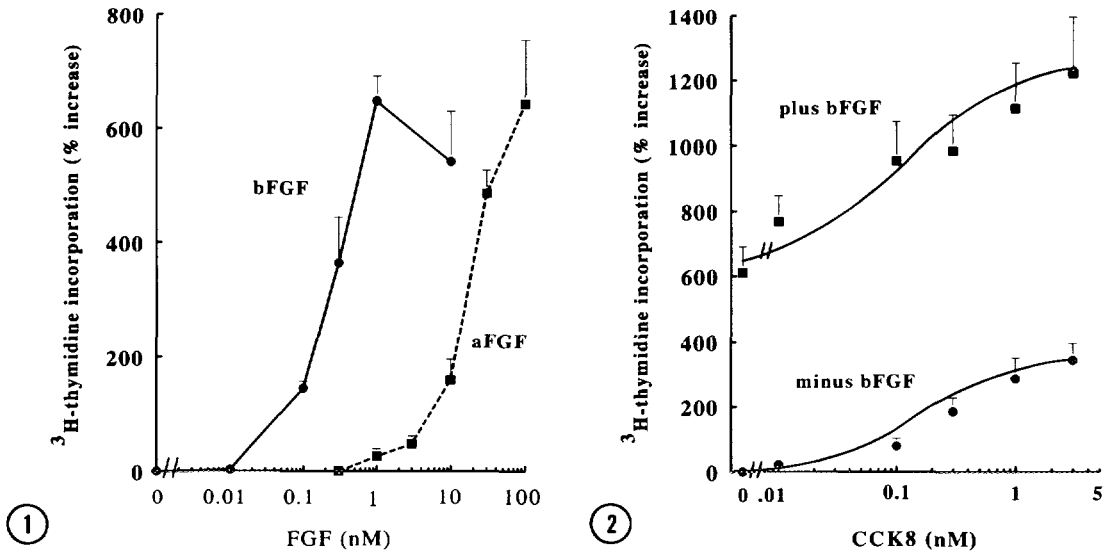
### **[<sup>3</sup>H]thymidine Incorporation**

DNA synthesis was estimated by measurement of [<sup>3</sup>H]thymidine incorporation into trichloroacetic acid-precipitable material. After 48h incubation, [<sup>3</sup>H]thymidine was added to each well (final concentration, 1 mCi/ml). Acini were incubated for an additional 24 h, then the media containing [<sup>3</sup>H]thymidine was removed. Acini were removed by scraping and sonicated in 0.5 ml of water with a probe type sonicator. Each sample was precipitated with trichloroacetic acid (TCA; final conc. 10%) at 4°C. The precipitates were washed twice with additional cold 10% TCA and dissolved in 0.5 ml of 0.1N NaOH. Radioactivity was measured by liquid scintillation counting. Measurements were made in triplicate wells. Results are expressed as the percentage of counts in the control wells or the percentage increase in counts compared to control wells and are means ± SE for 3-7 experiments.

## RESULTS AND DISCUSSION

### **Effect of basic and acidic FGF on pancreatic acini in vitro**

Previous studies indicated that basic FGF was capable of stimulating amylase release from rat pancreatic acinar cells (6). However, it is unlikely that FGF acts as a physiologic secretagogue as there is no known mechanism for FGF release by food ingestion and pancreatic secretion can be completely explained by the effects of acetylcholine and CCK (8). A more plausible role for FGF in the pancreas would be as



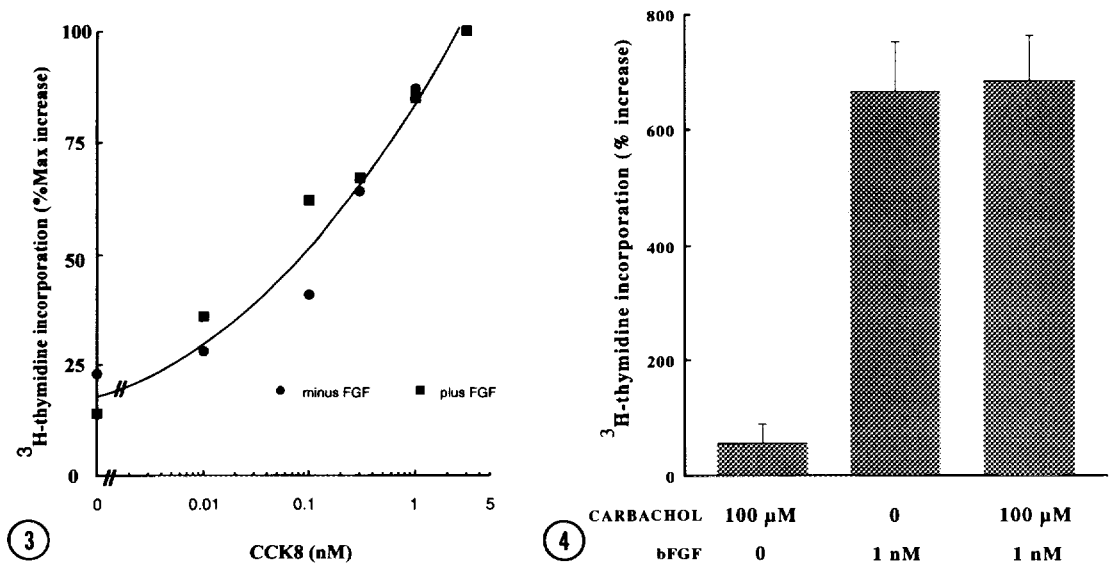
**Fig. 1.** Concentration dependence of FGFs on rat acinar cell DNA synthesis in vitro. Cells were cultured in the presence of the indicated dose of FGF for 3 days, the last 24 h of which included 1 mCi of [<sup>3</sup>H]thymidine. The solid and dotted lines show the effects of bFGF and aFGF, respectively. Results are expressed as the percent increase over incorporation measured in cultures without FGFs and are means  $\pm$  SE of means for 5 and 3 experiments for bFGF and aFGF, respectively.

**Fig. 2.** Effect of CCK8 with or without bFGF on rat acinar cell DNA synthesis in vitro. Acini were stimulated with 1 nM bFGF and various doses of CCK8 were added in combination. Cells were cultured for 3 days, the last 24 h of which included 1 mCi of [<sup>3</sup>H]thymidine. Results are expressed as the percent increase over incorporation measured in control cultures and are means  $\pm$  SE for 5 and 3 experiments for CCK8 alone and in combination, respectively.

a growth factor. To investigate the growth effects of FGFs, their effects on DNA synthesis of rat pancreatic acinar cells in primary culture were examined. The primary culture system has been previously described for rat pancreatic acinar cells (9) and is a modification of the system developed for mouse acinar cells (7). In this primary culture system both basic and acidic FGFs caused a dose-dependent increase in [<sup>3</sup>H]thymidine incorporation into DNA (Fig. 1) and caused a dramatic increase in cell spreading (not shown). Statistically significant effects were seen with 100 pM bFGF and 30 nM of aFGF and maximum effects were observed with 1 nM bFGF ( $747 \pm 44\%$  of control,  $n=15$   $p<0.01$ ) and with 100 nM aFGF ( $742 \pm 114\%$  of control,  $n=3$   $p<0.01$ ). Dose-dependency studies showed that aFGF was equally efficacious as bFGF but was 10 fold less potent (Fig. 1). These data on rat pancreatic acinar cells are compatible with previous studies that have suggested that bFGF is more potent than aFGF to induce growth responses in various cell lines in vitro (10).

### ***Effect of combinations of bFGF with CCK and carbachol on pancreatic acini in vitro***

Since FGFs were found to be potent stimulators of pancreatic acinar cell growth, their interactions with the known pancreatic trophic factor, cholecystokinin (CCK8) was examined. The effects of CCK8 and bFGF were tested individually and in combination on DNA synthesis in the primary cultures (Fig. 2). A maximal stimulatory dose of CCK8 lead to a  $264 \pm 42\%$  ( $n=16$ ) increase over control in [ $^3\text{H}$ ]thymidine incorporation. Interestingly, in the same cultures the effect of bFGF was always larger than that of a maximal CCK8 effect. Thus, bFGF is the most efficacious stimulant of acinar cell DNA synthesis in vitro tested to date. This observation suggests that FGFs might have an important role in regulation of pancreatic growth in vivo. When tested in combination the effects of maximal doses of bFGF and CCK8 was a 12-fold increase over control in [ $^3\text{H}$ ]thymidine incorporation ( $1247 \pm 170$ ,  $n=6$ ) (Fig. 2). Thus, the combined effect of the two stimulants was additive. It was also observed that the dose-dependency of CCK8 on [ $^3\text{H}$ ]thymidine incorporation was unchanged by the presence of bFGF (Fig.3).



**Fig. 3.** Concentration dependence of CCK8 stimulated DNA synthesis in cultures treated with and with-out bFGF. In order to more easily determine the effects of bFGF treatment on the dose-dependency of CCK8 induced stimulation of DNA synthesis in acinar cell cultures the data from Fig. 2 have been expressed as percent maximum CCK induced increase. This was accomplished by subtracting the values bFGF alone from from those of the combined stimulants and expressing the results as a percentage of the maximal increase.

**Fig. 4.** Effects of bFGF and carbachol on rat acinar cell DNA synthesis in vitro. Acini were stimulated with  $100 \mu\text{M}$  carbachol,  $1 \text{ nM}$  bFGF, or a combination of both. Cells were cultured for 3 days, the last 24 h of which included  $1 \text{ mCi}$  of [ $^3\text{H}$ ]thymidine. Results are expressed as the percentage increase in incorporation compared to control cultures and are means  $\pm$  SE for 3 experiments.

The mechanisms which account for the interactions between bFGF and CCK are not completely understood. One possible model to explain the observed additivity involves activation by a "common" second messenger. In this model each substance activates a critical second messenger, however, a maximum dose of each substance is not capable of attaining a maximal level of the second messenger and the effect of a combination on the pathway is additive. The identity of the "common" second messengers which might be involved in this interaction between CCK and FGF is unknown. The FGF receptor is a tyrosine kinase which activates several different sets of second messengers (11). These second messengers include: phospholipase C $\gamma$ , type I phosphoinositide-3-kinase; GAP, the GTPase-activating protein of c-ras; and, the serine/threonine kinases c-Raf and MAP kinase. The well known signal transduction pathways activated by CCK are those involved in the ability of CCK to activate cell secretion and include the breakdown of phosphoinositides resulting in the formation of inositol 1,4,5-trisphosphate and diacylglycerol, and leading to the mobilization of cellular Ca<sup>2+</sup> and the activation of protein kinase-C(12). Therefore, the activation of phospholipase C, mobilization of Ca<sup>2+</sup>, and activation of protein kinase C could be "common" pathways involved in the observed additive interaction. However, it was previously shown that the increase of inositol 1,4,5-trisphosphate by bFGF was only about half of that of carbachol, a pancreatic secretagogue which acts on secretion through pathways similar to CCK. Furthermore, there was no additive effect on the increase of inositol 1,4,5-trisphosphate with a combination of carbachol and bFGF(6). Thus, there is a discrepancy between the smaller effects of FGF on these phospholipase activated pathways and its larger effect on DNA synthesis. Also, several previous studies have reported that activation of phospholipase C $\gamma$  and protein kinase-C by FGF are not important for the stimulation of cell growth in different cell lines (13-15).

However, to further examine the possibility that common effects on the phospholipase C pathways accounted for the additivity of the effects of bFGF and CCK8 we tested the effects of bFGF in combination with the secretagogue carbachol. Carbachol itself had no effect on acinar cell DNA synthesis *in vitro*, nor did carbachol significantly potentiate the effects of bFGF (Figure 4). These data confirm in the rat our previous observation that carbachol was not a trophic factor for mouse pancreatic acini (7). Furthermore, these data strongly argue against a role for the signal transduction pathways involved in secretion as being important for growth regulation in the pancreatic acinar cells.

A number of G protein-coupled receptor agonists such as angiotensin, vasopressin, and bombesin have previously been shown to stimulate tyrosine phosphorylation (16-18). Recent evidence suggests that CCK also stimulates tyrosine phosphorylation in pancreatic acinar cells (19). Therefore, it is possible that CCK8 and bFGF stimulate DNA synthesis through different but converging intracellular pathways involving tyrosine kinases. Of interest is the observation that the

patterns of protein tyrosine phosphorylation by the receptor tyrosine kinases and the G protein-coupled receptor families appear to be different (18). Further investigations will be necessary to identify the mechanisms involved in pancreatic acinar cell growth regulation. However, the data reported here strongly indicate that FGF should be considered an acinar cell trophic factor.

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