Mediation of Basic Fibroblast Growth Factor-Induced Lactotrophic Cell Proliferation by Src-Ras-Mitogen-Activated Protein Kinase p44/42 Signaling

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Basic fibroblast growth factor (bFGF), which is secreted from folliculostellate cells in the anterior pituitary, is known to be involved in the communication between folliculostellate cells and lactotropes during estradiol-induced lactotrope cell proliferation. We studied the role of MAPK p44/42 in bFGF-regulated cell proliferation using enriched lactotropes and the lactotrope-derived PR1 cell line. In cell cultures, bFGF increased cell proliferation of PR1 cells and enriched lactotropes. In both of these cell populations, bFGF also increased phosphorylation of MAPK p44/42. U0126, an inhibitor of MAPK p44/42, blocked the bFGF-induced activation of MAPK p44/42 as well as the bFGF-induced cell proliferation of enriched lactotropes and PR1 cells. Treatment of PR1 cells with bFGF increased the activity of Ras p21, whereas overexpression of a dominant negative mutant of Ras p21 abrogated the bFGF-induced activation of MAPK p44/42 in these cells. Furthermore, the Src kinase inhibitor PP1 suppressed bFGF-induced activation of MAPK p44/42 in both enriched lactotropes and PR1 cells. The Src kinase inhibitor PP1 also reduced bFGF activation of Ras p21 and cell proliferation in PR1 cells. On the other hand, the bFGF-induced activation of MAPK p44/42 in enriched lactotropes and PR1 cells was not affected by protein kinase C inhibitors. These data suggest that bFGF induction of lactotrope cell proliferation is possibly mediated by activation of Src kinase, Ras p21, and MAPK p44/42. (Endocrinology 146: 1948–1955, 2005)

THE PITUITARY is the site of synthesis, as well as the target, of several growth factors that modulate hormone production and regulate pituitary cell growth. Growth factors have been implicated in pituitary tumorigenesis. Basic fibroblast growth factor (bFGF) was originally isolated from bovine pituitary (1) and is differentially expressed by pituitary adenoma cells, with higher levels noted in more aggressive tumors (2). In the human pituitary, bFGF is produced by adenohypophysial cells that comprise pituitary adenomas (3, 4). In the anterior pituitary, folliculostellate (FS) cells are the primary cells that produce bFGF (5). We have recently demonstrated that FS cells participate in estrogen’s mitogenic action on lactotropes (6). Estradiol induces the release of TGF-β3 from lactotropes; TGF-β3 acts on FS cells to release bFGF, which then acts on lactotropes to increase cell proliferation. Thus, by releasing bFGF, FS cells play an important role in mediating estradiol’s mitogenic action on lactotropes.

bFGF is a member of a large group of fibroblast growth factor (FGF) peptides that control multiple cellular processes, including cell migration, survival, proliferation, and differentiation (7). FGF peptides mediate their biological responses by binding to and activating a family of receptor tyrosine kinases designated as FGF receptors (FGFRs) (8, 9). The peptide bFGF possesses a dual receptor system consisting of tyrosine kinase FGFRs and heparin sulfate proteoglycans. It has been found that, in most cases, both of these components must be present in order for bFGF to trigger proliferation (10, 11). Binding of bFGF to the receptor is known to cause receptor dimerization and activation of the cytoplasmic domain. These receptor tyrosine kinases subsequently activate multiple signaling pathways, including protein kinase C (PKC), Src kinases, Ras, and MAPK p44/42 (12–15). PKC, a member of the kinase family, can activate MAPK p44/42 in a Ras-dependent or -independent manner (16, 17).

FGF-2 has been shown to activate prolactin gene transcription through a PKC-activated, but Ras-independent, MAPK p44/42 pathway in GH4C1 pituitary tumor cells (18). bFGF is known to activate Src kinases, which can further result in the activation of MAPK p44/42 via a Ras-dependent or -independent pathway, depending on the cell system (19–21). bFGF has been shown to stimulate prolactin secretion and proliferation of pituitary lactotropic cells (6, 18, 22). Although the mechanism by which bFGF increases prolactin secretion has been studied (18), currently there are no reports of the signaling mechanism’s involvement in bFGF action on lactotrophic cell proliferation. In this study, we employed enriched lactotropes and the lactotrophic cell-derived PR1 cell line to determine the role of MAPK p44/42 in bFGF’s action on cell proliferation. Using inhibitors for various kinases, we found that activation of the family of Src kinases and activation of Ras p21 are prerequisites for bFGF-induced MAPK p44/42 activation and the proliferation of PR1 cells.

Materials and Methods

Enrichment of lactotropes

Anterior pituitary tissues from female Fischer-344 rats (150–175 g) were collected and used for preparation of enriched lactotropes. Lac-
trophotropes were enriched from dissociated anterior pituitary cell suspensions using a discontinuous Percoll gradient as described before (6, 23). Briefly, cells were dissociated and layered, using a 1-ml plastic pipette, on top of the Percoll (Sigma, St. Louis, MO) gradient consisting of 60, 50, and 35% Percoll layers. The gradient was centrifuged at 450 × g for 20 min. The cells at the 35%/50% interface were collected with a glass pipette and seeded on poly-L-lysine-coated 96-well plates (Becton Dickinson, San Jose, CA) as enriched lactotropes. The cells were maintained in phenol-red free DMEM/F-12 with 10% fetal calf serum (FCS; HyClone Laboratories, Inc., Logan, UT) for 1 d, and then in medium containing 2.5% FCS and 10% horse serum (HyClone) for 1 more day. During drug treatment, cells were maintained in phenol-red free DMEM/F-12 containing serum supplement (30 nM selenium, 1 μM iron-free human transferrin, 100 μM putrescine, and 5 μg/ml insulin) and estradiol (10 nM). This culture condition has been shown to maximize the bFGF effects on lactotrope cell proliferation (6). Enriched lactotropes were maintained in medium containing serum supplement and estradiol for 24 h and were then treated with bFGF for 72 h in the presence and absence of MEK inhibitor (U0126), before assaying for [3H]thymidine uptake or instead treated with bFGF for 1 h for MAPK p44/42 phosphorylation.

**PR1 cell culture and reagents**

The PR1 cell line is derived from an estrogen-induced prolactinoma in a Fischer-344 rat. Like lactotropes, PR1 cells secrete prolactin and respond positively to bFGF and estradiol (6, 24, 25). Because lactotrope enrichment requires a large number of animals to get enough pituitaries, and because PR1 cells show a growth response to bFGF similar to that of lactotropes (Fig. 1), we primarily used PR1 cells to determine the cellular mechanism of bFGF action on cell proliferation. Additionally, unlike lactotropes (6), PR1 cells do not require estradiol for growth initiation; therefore, these cells provide a cellular model for determining direct action of bFGF on cell growth (Fig. 1). However, we have also conducted studies to confirm some of the major observations of PR1 cells in enriched lactotropes. PR1 cells were maintained in phenol-red free DMEM/F12 media with 10% FCS. During experimentation, cells were maintained in serum supplement (30 nM selenium, 1 μM iron-free human transferrin, 100 μM putrescine, and 5 μg/ml insulin) and estradiol (10 nM). This culture condition has been shown to maximize the bFGF effects on lactotrope cell proliferation (6). Enriched lactotropes were maintained in medium containing serum supplement and estradiol for 24 h and were then treated with bFGF for 72 h in the presence and absence of MEK inhibitor (U0126), before assaying for [3H]thymidine uptake or instead treated with bFGF for 1 h for MAPK p44/42 phosphorylation.

**[3H]Thymidine uptake assay and cell counting**

PR1 cells (25,000/well) or enriched lactotropes (50,000/well) were grown in 96-well plates in cultures as just described. Cells were treated with or without various concentrations of bFGF (1–10 ng/ml) for various time periods as indicated in the figures. The cultures were treated with 0.5 μCi [methyl-3H]thymidine (specific activity, 82.2 Ci/mM; Amersham Corp., Arlington Heights, IL) per well for 8 h before harvesting. Cells were harvested using a cell harvester (Packard Bioscience, Meriden, CT) and counted in a liquid scintillation counter. For cell counting experiments, cells (100,000/well) were grown in 24-well plates and treated, as just described, with bFGF (10 ng/ml) for various time periods. After each time period, cells were dissociated using trypsin-EDTA, and cell viability was confirmed with the Trypan blue exclusion method. The percentage of viable cells was calculated as unstained cells/total number of cells × 100, and data are presented as a percentage of the control. For all the blocking studies, cells were pretreated with the inhibitor for 1 h, followed by bFGF (10 ng/ml) treatment in the presence of the inhibitor for various time periods. Medium was changed every 48 h. Control cells were treated with the vehicle (dimethylsulfoxide).

**Western blot**

PR1 cells (0.5 million/well) were grown in six-well plates followed by incubation in media containing serum supplement. Cells were then treated with or without bFGF (10 ng/ml) for various time periods. Cells were lysed and then analyzed for total and phosphorylated MAPK p44/42 by immunoblotting. Monoclonal and polyclonal antibodies, specific for phosphorylated MAPK p44/42 (phospho-MAPK p44/42) and the Src kinase inhibitor PP1 were all purchased from Calbiochem (San Diego, CA). We bought bFGF from R&D Systems (Minneapolis, MN), a plasmid containing a dominant negative Ras (Ras N17) from Clontech Laboratories (Palo Alto, CA), and a Ras activation assay kit from Upstate Biotechnology.

**Fig. 1.** bFGF-induced proliferation of PR1 cells. A, PR1 cells were treated with various doses of bFGF (0–10 ng/ml) for 48 h, and the uptake of [3H]thymidine in these cells was determined. The results are presented in counts-per-minute values. Each bar represents mean ± SEM of three separate experiments. a, P < 0.05, significantly different from the zero-dose (control); b, P < 0.05, significantly different from the 1 ng/ml-treated group. B, Showing the effect of bFGF on [3H]thymidine uptake in PR1 cells at various time intervals. Data are presented as a percentage of the control group (cpm values for 24 h, 48 h, and 72 h were: 5,641 ± 227, 8,744 ± 175, and 13,666 ± 406, respectively; n = 6). C, Showing the change in cell counts after bFGF treatment at various time intervals. a, P < 0.01, significantly different from the respective control groups; b, P < 0.01, significantly different from bFGF treatment at 24 h; c, P < 0.01, significantly different from bFGF treatment at 48 h; n = 3.
phosphatase inhibitors I and II (Sigma). The cellular debris was cleared by centrifugation (10,000 × g for 10 min). The protein concentration in the supernatant was determined using a Bio-Rad protein assay reagent. Equal amounts of protein from each sample were resolved on SDS-PAGE and transferred to Immobilon-P polyvinylidene difluoride membranes (Millipore, Bedford, MA). Total MAPK p44/42 and phospho-MAPK p44/42 proteins were detected together in one blot at the same time. For this purpose, membranes were incubated with both rabbit anti-MAPK p44/42 and mouse antiphospho-MAPK p44/42 antibody in blocking buffer (Licor Biotechnology, Lincoln, NE). Afterward, membranes were washed and incubated with fluorescent-labeled antimouse and antirabbit antibody together for 1 h. Membranes were washed and scanned at 700 nm (antirabbit) and 800 nm (antimouse) wavelengths using an Infrared Imaging System (Licor Biotechnology) to determine the fluorescence intensity of the bands for phospho- and total MAPK p44/42. These fluorescent images were converted to gray images using the Infrared Imaging System program as shown in all the figures.

For quantification of MAPK p44/42 activity, band intensities of phospho-MAPK p44/42 were determined using Scion Image software (Scion Corp., Frederick, MD) and then normalized to the corresponding total MAPK p44/42.

**Transient transfection**

In 24-well plates, PRI1 cells were transfected with a plasmid-encoding dominant negative mutant of Ras N17 or a vector plasmid using the LipofectAMINE 2000 reagent (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. After 24 h, cells were treated with or without bFGF (10 ng/ml) for 1 h. Cells were lysed and then analyzed for total and phospho-MAPK p44/42 by immunoblotting.

**Ras activity assay**

PRI1 cells, numbering 5 × 10^5 per treatment, were stimulated with bFGF (10 ng/ml) for various time periods and lysed for 30 min on ice in 500 µl lysis buffer provided with the Ras activation assay kit. The lysates were then centrifuged, and supernatants were mixed with 30 µl glutathione-S-transferase fusion protein containing the Ras binding domain of Raf, and immobilized in glutathione-agarose beads. Samples were incubated for 90 min at 4°C with gentle rotation. The beads were then washed three times with lysis buffer. The GTP-bound Ras p21 protein was eluted in gel-loading buffer and subjected to 12.5% SDS-PAGE and then transferred to Immobilon-P polyvinylidene difluoride membranes. The levels of active Ras were assessed by immunoblotting with anti-Ras antibody provided with the aforementioned kit. Briefly, membranes were incubated with anti-Ras antibody for 1 h at room temperature in 5% milk, 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.1% Tween 20. Membranes were washed and incubated with peroxi-
dase-conjugated antiamouse antibody for 1 h; they were then developed using an ECL Western blot chemiluminescence reagent (Amersham Biosciences, Piscataway, NJ). For positive and negative controls, untreated cell lysates were loaded with GTPγS or GDP, respectively, before mixing with beads per the kit’s instructions.

**Statistical analysis**

Data shown in figures are mean ± SEM of the indicated number of experiments performed independently. Data were analyzed using one-way ANOVA for comparisons among groups. Post hoc analysis, after ANOVA, employed the Newman-Keuls test. Two-way ANOVA was used to compare the differences between various treatments at various time intervals. The Bonferroni post-test was used to compare differences in control and bFGF-treated groups at various time intervals. A probability value less than 0.05 was considered significant.

**Results**

**Effect of bFGF on cell proliferation**

We have previously shown that primary cultures of enriched lactotropes demonstrate increased cell proliferation when treated with bFGF (6). Although PRI1 cells are lactotrope-derived, the effect of bFGF on the proliferation of PRI1 cells has not been determined. We therefore examined the effect of the growth factor on 3[H]thymidine uptake in these cells. As shown in Fig. 1A, exposure of PRI1 cells to various concentrations (0–10 ng/ml) of bFGF for 48 h resulted in a concentration-dependent increase in 3[H]thymidine uptake in these cells, suggesting that the peptide concentration-dependently increased the proliferation of PRI1 cells. The kinetic study of the bFGF-induced proliferation showed significant proliferation of PRI1 cells at 24 h and a time-dependent increase until 72 h (Fig. 1B). Determination of the number of viable cells after treatment with bFGF or control for various time periods also indicated a time-dependent cell growth effect of the growth factor (Fig. 1C). The bFGF cell growth response of the PRI1 cells appears to be similar to those observed in enriched lactotropes (Ref. 6 and see Fig. 3B).

**Effects on MAPK p44/42**

Several lines of evidence suggest a role of MAPK p44/42 in the regulation of cell proliferation by various growth factors (26). To determine whether this kinase plays a role in bFGF-induced PRI1 cell proliferation, we studied the effect of bFGF on MAPK p44/42 activation in PRI1 cells. bFGF was used at a concentration of 10 ng/ml, a level at which we had seen the highest growth stimulation of PRI1 cells. After bFGF was added to the culture, we determined MAPK p44/42 activation, at various time intervals, using Western blot with a phospho-specific antibody (Fig. 2A). Densitometric analysis of the blots demonstrated that bFGF time-dependently increased phosphorylation of MAPK p44/42 between 0.25 and 12 h, with peak activity achieved at 1–2 h (Fig. 2B). Persistent activation of MAPK p44/42 was observed up to 12 h. Untreated control groups didn’t show any basal activation at 0, 4, or 12 h (data not shown).

**Effects of a MAPK p44/42 inhibitor**

Because bFGF activated MAPK p44/42 and the proliferation of PRI1 cells, we subsequently tested whether activation of MAPK p44/42 regulates growth factor-induced cell proliferation. To test this, we evaluated the effect of U0126, an inhibitor of the MAPK p44/42 pathway, which is known to block the function of MEK1/2 kinase (27), on bFGF-induced PRI1 cell proliferation. MEK1/2 kinase is an upstream activator of MAPK p44/42, and it controls the dual phosphorylation of specific threonine and tyrosine residues (Thr 183 and Tyr 185) on MAPK p44/42 (28, 29). Previously we had shown that a 10-µM dose of U0126 significantly affected phosphorylation of MAPK p44/42 in pituitary-derived cells (30). Here we showed that the inhibitor blocked bFGF-activated phosphorylation of MAPK p44/42 in PRI1 cells in a dose-dependent manner, with maximum inhibition at the 10 µM concentration (Fig. 2, C and D). The highest dose of the inhibitor also blocked the effect of bFGF on 3[H]thymidine uptake in PRI1 cells at various time periods (Fig. 2E). U0126 has been also shown, by Caporali et al. (24), to block MAPK activation in PRI1 cells. The basal proliferation of PRI1 cells, as determined by 3[H]thymidine uptake, was not inhibited by U0126 at this concentration [cpm values after 48 h: control, 8,744 ± 175; U0126 (10 µM), 7,975 ± 367; values after 72 h:...
Fig. 2. Kinetics of bFGF-induced phosphorylation of MAPK p44/42. PR1 cells were treated with bFGF (10 ng/ml) for various time periods. Cell extracts were collected and used to determine MAPK p44/42 phosphorylation by Western blotting. A, Representative blot showing kinetics of MAPK p44/42 activation. B, Densitometry analysis of phosphorylated vs. total MAPK p44/42. Data express the fold change over control cells. a, P < 0.01, significantly different from control group; b, P < 0.05, significantly different from those treated for 0.25 h and 0.5 h; c, P < 0.05, significantly different from those treated for 1 h and 2 h. C and D, Effects of MEK1/2 inhibitor U0126 on bFGF-induced MAPK p44/42 activation. PR1 cells were pretreated with U0126 for 1 h, followed by bFGF (10 ng/ml) treatment for another 1 h to determine MAPK p44/42 phosphorylation by Western blotting. C, Representative blots showing the effect of U0126 on MAPK p44/42 activation. D, Densitometric analysis of phosphorylated vs. total MAPK p44/42. The data express the fold change over controls. a, P < 0.05, significantly different from respective controls; b, P < 0.001, significantly different from all other groups; c, P < 0.001, significantly different from the 0.1 μM U0126 + bFGF group; d, P < 0.001, significantly different from the 1 μM U0126 + bFGF group. E, Effect of U0126 on bFGF-induced incorporation of [3H] thymidine into PR1 cells. Results are presented as a percentage of the control group. a, P < 0.05, significantly different from respective controls; b, P < 0.05, significantly different from respective bFGF-treated groups. Each bar represents the mean ± SEM of four to six independent experiments.

control, 13,656 ± 403; U0126 (10 μM), 13,654 ± 640 (n = 6)]. These results suggest that bFGF may activate the MAPK p44/42 pathway to regulate PR1 cell proliferation.

Whether MAPK p44/42 is also activated by bFGF in primary lactotropes was tested. For this purpose, we examined the effects of bFGF, in the presence and absence of U0126, on enriched lactotropes in primary cultures. As shown in Fig. 3A, similar to PR1 cells, bFGF activated MAPK p44/42 in enriched lactotropes, and U0126 abrogated the effect of bFGF on MAPK p44/42 activation (phosphorylation of MAPK p44/42 fold change: control, 1.09 ± 0.11; bFGF, 2.6 ± 0.21; U0126, 1.31 ± 0.17; U0126 + bFGF, 1.33 ± 0.276; n = 3). There were no significant differences among control, U0126, and U0126 + bFGF groups; however, the bFGF-treated group (P < 0.01) was significantly different from all other groups. bFGF also increased the proliferation of lactotropes, as reported by us earlier (6), that was suppressed by U0126 (Fig. 3B). These results suggest that bFGF-induced cell proliferation in both PR1 cells and enriched lactotropes is dependent on activation of MAPK p44/42.

**Effects of an Src kinase inhibitor**

Previously it has been shown that the Src family of protein kinases participates in the mediation of MAPK action on cell proliferation (31). To investigate the role of Src kinases in bFGF-induced MAPK p44/42 activation and cell proliferation, we determined the effect of the Src kinase inhibitor PP1 on bFGF-induced activation of MAPK p44/42 and cell proliferation. PP1 at 5–20 μM concentrations showed a concentration-dependent inhibition of MAPK p44/42 phosphorylation (Fig. 4, A and B). The 20 μM concentration of PP1, which blocked the bFGF effect on MAPK, also suppressed the bFGF effect on [3H] thymidine uptake in PR1 cells at various time intervals (Fig. 4C). This concentration, which conforms to that of other studies in Src activity blocking (28), did not affect the basal [3H] thymidine uptake in PR1 cells [cpm values after 48 h: control, 8,744 ± 175; PP1 (20 μM), 8,346 ± 215; and after 72 h: control, 13,636 ± 403; PP1 (20 μM), 12,340 ± 640 (n = 6)]. This inhibitor has been also shown to inhibit Src kinase activity in PR1 cells (24).

Similar to PR1 cells, in enriched lactotropes the activation of MAPK p44/42 by bFGF was reversed by PP1 [levels of phospho-MAPK p44/42 fold change: control, 1.05 ± 0.15; bFGF, 2.47 ± 0.27; PP1, 1.03 ± 0.01; PP1 + bFGF, 1.32 ± 0.128 (n = 3–5)]. There were no significant differences among control, PP1, and PP1 + bFGF groups; however, the bFGF-treated group (P < 0.05) was significantly different from all other groups. These data suggest that the Src family of protein kinases may be involved in bFGF-activated MAPK p44/42-regulated PR1 and lactotropic cell proliferation.

**Effect of bFGF on Ras p21 activation**

Ras p21, a member of the family of small G proteins, is activated by Src kinase and is known to be involved in the
phosphorylation of MAPK p44/42 (20, 21, 28). Whether the Src-Ras-MAPK pathway is involved in bFGF action on PR1 cells was tested. We determined the effect of bFGF, with and without the Src blocker PP1, on Ras p21 activation. We evaluated the levels of active (GTP-bound) Ras p21 by pull-down experiments, described by us previously, using an immobilized glutathione-S-transferase fusion protein containing the binding domain of Raf (30). To validate our technique, untreated lysates of PR1 cells were loaded with GTP/H9253/S or GDP, for positive and negative controls, respectively, before mixing with beads as per manufacturer instructions (Fig. 5A).

As shown in Fig. 5A, bFGF increased the amount of active GTP-Ras p21 in PR1 cells. The Src kinase inhibitor PP1 decreased the activation of Ras p21 at various time intervals, suggesting that the activation of Src kinase is required for Ras activity. To evaluate whether activation of Ras plays a role in bFGF activation of MAPK p44/42, we examined the effect of transient overexpression of Ras N17, a dominant negative mutant of Ras p21, on bFGF-induced activation of MAPK p44/42. Previously, expression of Ras N17 has been shown to yield an inhibitory effect on endogenous Ras (32, 33). Overexpression of dominant negative Ras has been shown to suppress Ras activity in GHFT, immortalized pituitary cells (34). In this study, we found that overexpression of Ras N17 in PR1 cells reduced the activation of MAPK p44/42 increased by bFGF (Fig. 5B). These results suggest that bFGF-induced Ras activity may be required for the phosphorylation of MAPK p44/42 in PR1 cells.
Effects of PKC inhibitor

In several cells, when bFGF binds to the receptor, this activates PKC activity (35, 36), which can further activate MAPK p44/42 via Ras activation (37, 38). To identify whether such interaction occurs in PR1 cells, we evaluated the effect of PKC inhibitors on bFGF-induced MAPK p44/42 activation and 3[H]thymidine uptake in PR1 cells. As shown in Fig. 6, treatment with the PKC inhibitor ChCl2 (1.25–5 μM) did not show any effect on bFGF-induced MAPK p44/42 phosphorylation and 3[H]thymidine uptake in PR1 cells. These concentrations of ChCl2 are known to inhibit PKC activation and almost all PKC isoforms (39–41). This inhibitor alone did not show any effect on the growth of the cells.
as detected by \[^{3}H\]thymidine uptake [cpm values after 48 h: control, 8,744 ± 175; ChCl\(_2\) (5 μM), 7,648 ± 473; and after 72 h: control, 13,636 ± 403; ChCl\(_2\) (5 μM), 12,399 ± 700 (n = 5–6)]. To confirm our data, we used Bisindolylmaleimide, which is known to block the effect of PKC in pituitary cells (30, 35) at a 1–5 μM concentration. Similar to ChCl\(_2\), this inhibitor did not show any effect on bFGF-induced MAPK activation (data not shown). Further, the PKC-inhibitor ChCl\(_2\) did not affect the activation of MAPK p44/42 by bFGF in primary cultures of enriched lactotropes [levels of phospho-MAPK p44/42fold change: control, 1.05 ± 0.15; bFGF, 2.47 ± 0.27; ChCl\(_2\), 1.02 ± 0.04; ChCl\(_2\) + bFGF, 3.09 ± 0.33 (n = 3–5)]. There was no significant difference between the bFGF + inhibitor group and the bFGF-only group. These data suggest that the bFGF-induced proliferation of lactotropes does not involve PKC activation.

**Discussion**

The data presented here demonstrate that bFGF induces proliferation of PR1 cells by activating MAPK p44/42 phosphorylation in both PR1 cells and enriched lactotropes. We also showed that the Src kinase inhibitor PP1, in the presence of bFGF, blocked not only phosphorylation of MAPK p44/42 but also the proliferation of PR1 cells. Furthermore, bFGF increased the activation of Ras p21, whereas PP1 inhibited this bFGF-induced activation. In addition, overexpression of a dominant negative mutant of Ras p21 blocked the activation of MAPK p44/42 by bFGF. PKC, known to be involved in bFGF-induced prolactin gene expression, was not found to be involved in bFGF-induced MAPK p44/42 activation and cell proliferation in PR1 cells and enriched lactotropes. These data suggest that bFGF increases lactotrop cell proliferation via PKC-independent Src-Ras-MEK-MAPK p44/42 signaling.

Pituitary-derived bFGF has been shown to stimulate the proliferation of prolactin-secreting cells (42). High bFGF activity has been documented in patients with sporadic pituitary adenomas (2). Previously it has been shown that bFGF mediates estradiol’s mitogenic action on lactotropes (6, 30, 42). This data set is consistent with the finding that bFGF acts as a lactotrophic cell growth-regulating factor. FGF-related peptides are known to bind and phosphorylate FGFRs (8, 9). FGFRs have been shown to activate the Ras-MAPK p44/42 pathway via an FGFR docking protein (43). This signaling cascade can result in diverse biological responses such as proliferation, cytoskeletal reorganization, migration, differentiation, survival, and apoptosis (13). Activated FGFRs can also activate phospholipase C-γ, which hydrolyzes phosphotidylinositol bis phosphate into diacylglycerol and inositol triphosphate, leading to the stimulation of PKC (14, 44). Reports from various groups have suggested a role of PKC-induced MAPK p44/42 in nonmitogenic effects of FGFs (45). FGF-activated prolactin gene transcription has been shown to be mediated by PKC in GH4T2 pituitary tumor cells (18). However, we have not found any effect of PKC inhibitors on bFGF-induced proliferation of PR1 cells or enriched lactotropes. It is interesting to note that the activation of prolactin gene expression by estradiol in PR1 and GH3 somatolactotroph cell lines is mediated by MAPK p44/42 but is independent of Src (46). However, estradiol-induced cell survival of PR1 cells uses an Src-MAPK p44/42-dependent pathway (24).

Src kinases belong to a group of cytoplasmic kinases that can be activated by FGFRs via tyrosine kinases (47). Based on the data presented here, it appears that MAPK p44/42 phosphorylation via the activation of Src kinases is required for cell growth or survival, whereas an Src kinase-independent, but PKC-dependent, MAPK pathway is required for prolactin gene expression in pituitary cells.

**Acknowledgments**

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This work was supported by National Institutes of Health Grants CA77550 and AA11591.

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Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.