Vol. 167, No. 1, 1990
February 28, 1990
BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 96-102

FIBROBLAST GROWTH FACTOR INDUCES β-AMYLOID PRECURSOR mRNA IN GLIAL BUT NOT NEURONAL CULTURED CELLS

Diana Quon, Rosanne Catalano and Barbara Cordell*

California Biotechnology, Inc.
2450 Bayshore Parkway, Mountain View, CA 94043

Received January 12, 1990

Treatment of PC12 and C6 cell cultures with recombinant basic fibroblast growth factor results in approximately a five to ten-fold stimulation of β-amyloid precursor mRNA in the C6 astrocytoma cell line but only a slight induction of precursor mRNA in the PC-12 neuronal cell line. Stimulation of expression occurred at a hormone concentration of ~0.5 to 1 nM and was seen after 2 days. These results suggest that basic fibroblast growth factor may contribute to amyloidosis of Alzheimer's disease.

Trophic factors have been shown to carry out important functions in neuronal development, maintenance of function, and regeneration. Basic fibroblast growth factor (bFGF) is a well-characterized mitogen (reviewed in ref. 1) which has also been demonstrated to possess neurotrophic effects in vitro and in vivo (reviewed in ref. 2,3). Using the PC-12 clone of a rat pheochromocytoma, several groups have demonstrated the ability of bFGF to stimulate neurite outgrowth (4-6), induce neural-specific mRNAs (5), and enzymatic activities such as acetylcholine esterase (4), ornithine decarboxylase (5), and choline acetyltransferase (7). In addition, bFGF appears to be an autocrine factor for astrocytes. bFGF is produced by astrocytes grown in vitro and can stimulate their proliferation (8). The hormone is also synthesized by macrophages (9). Experimental lesion of the adult rat brain results in increased bFGF immunoreactivity at the site of the wound (10), hence bFGF released at the site of injury may stimulate neuronal survival and regeneration of neurites. In this same setting bFGF may also promote gliosis.

The β-amyloid precursor protein (β-APP) contains a peptide sequence which gives rise to amyloid deposits unique to the brains of individuals with Alzheimer's disease (reviewed in ref. 11). The

*To whom correspondence should be addressed.

0006-291X/90 $1.50

amyloidogenic peptide resides on several distinct isoforms of β-APP (12-16) and it has been suggested that abnormal expression and/or processing of the precursors or one of its isoforms may account for amyloid deposition and plaque formation (11,12,17). However, the source of amyloid and the mechansism(s) leading to amyloid formation are unknown. To gain insight into the issues of amyloid genesis, we investigated bFGF as a potential influencing molecule on β-APP expression. We initated our study using two rat cultured cell lines, one of neuronal origin, PC-12; the other of glial origin, C6.

MATERIALS AND METHODS

Materials 7S Nerve growth factor (NGF) was purchased from Collaborative Research, Inc. (Bedford, MA). Human bFGF (154 amino acid form) was recombinantly produced in E. coli and purified to homogeneity using anion exchange chromatography. c-fos cDNA was obtained from the American Type Culture Collection. β-APP, β-actin cDNAs and the Kunitz inhibitor β-APP 751/770/563-specific oligonucleotide have been described (13). A rat β-APP 695-specific oligonucleotide (5'-GGCTGCCGTCGTGGGAACTCGGACTACCTCCTCC-3') was chemically synthesized.

Cell culture PC-12 cells were maintained as described by Greene and Tischler (18) using Dulbecco's minimal essential medium 21 supplemented with 1% glutamine and 5% each, heat inactivated horse and fetal bovine sera. C6 cells were purchased from the American Type Culture Collection (Rockville, MD) and were maintained as suggested in Ham's F10 medium supplemented with 15% horse serum and 2.5% fetal bovine serum. bFGF treatment of PC-12 and C6 cells was at 20 ng/ml and 10 ng/ml, respectively, in serum containing medium for approximately 48 hours. Treatment of PC-12 cells with NGF was at 100 ng/ml for 48 hours. Cells were at 50% confluency at the initiation of treatment. Phase contrast photomicrographs of treated cells were made with a Nikon microscope shot at an ASA of 800.

Isolation, fractionation and hybridization of RNA Total RNA was prepared from cell cultures according to the method of Chomczynski and Sacchi (19). 10μg of each RNA sample was fractionated by electrophoresis on 1.2% agarose gels containing formaldehyde. RNA was transferred to Hybond N+ membranes from Amersham Corp. (Arlington Hgts, IL) in 20X SSC followed by crosslinking with ultraviolet light. Location of 28S and 18S ribosomal RNAs was determined by staining with ethidium bromide. Radiolabeling of probes and hybridizations of both cDNAs and oligonucleotides were carried out as previously described (13). Quantitation of RNAs was made by tracing of the autoradiograms with a Shimadzu CS-930 scanning densitometer.

RESULTS AND DISCUSSION

We first documented the morphological changes of PC-12 cells previously shown to occur upon exposure to bFGF. The neuronal

differentiation produced by bFGF is similar, if not identical, to that seen from NGF treatment (4-6), therefore, NGF was used as a control. Identical cultures PC-12 cells were prepared; one was treated with 20 ng/ml (~1 nM) recombinant bFGF; another was treated with 100 ng/ml NGF; the last was untreated. The three cultures were incubated for approximately 48 hours afterwhich each culture was photographed then extracted for RNA. Figure 1 confirms that bFGF treatment of PC-12 cells induces morphological changes consistent with differentiation to a neuronal phenotype. The cells have lost their refractile property and have begun to elaborate numerous neuritic processes. The bFGF treated PC-12 cells also appear similar to those cells treated with NGF.

The RNA isolated from each PC-12 culture was examined for β-amyloid precursor protein (β-APP) mRNA. Northern blots hybridized with a β-APP cDNA probe revealed a broad, predominant RNA of 3.6-3.4 kilobases (kb), as well as a ~2 kb minor RNA species in all samples (Figure 2). The 3.6-3.4 kb mRNA corresponds to the transcript sizes typically observed for β-APP (13-15). The minor, lower molecular weight β-APP mRNA of ~2.0 kb

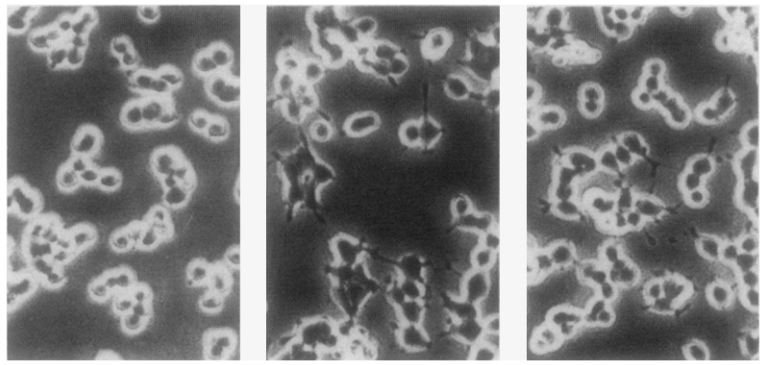
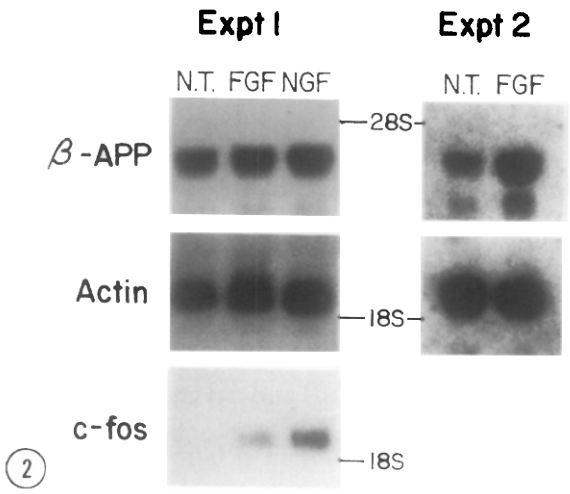


Figure 1 Morphological alterations of PC-12 cells induced after 48 hour incubation with 20 ng/ml bFGF (center panel) or 100 ng/ml NGF (right panel) as compared to untreated cells (NT left panel).

presumably encodes the 563 isoform of β-APP recently reported (16). Similar levels of β-APP RNA are noticed in cultures both treated with hormones and untreated suggesting a lack of mRNA induction by either bFGF or NGF under the conditions used here. Densitometric measurement of the autoradiograms confirmed that the β-APP expression was not substantially induced. To insure that equivalent amounts of RNA were applied to the gel and transfered, the membrane was stripped then hybridized with a β-actin cDNA probe. Equivalent hybridization was seen in all samples for β-actin mRNA (Figure 2). Further, we hybridized an identical blot with a c-fos probe as a positive control for hormone induction since it has been demonstrated by Greenberg et al. that the c-fos proto-oncogene is stimulated several-fold in PC-12 cells by bFGF or NGF (20). Indeed, we found the 2.2 kb c-fos mRNA level was stimulated by both bFGF and NGF (Figure 2). Several independent, identical experiments were performed assaying bFGF induction of β-APP transcripts. The maximum induction ever obtained was only two-fold (Figure 2, right panel). β-APP isoforms containing a domain homologous to the Kunitz family of serine proteinase inhibitors (β-APP 751/770) are ubiquitously expressed - unlike neuronal cells which also contain an isoform lacking this sequence (β-APP 695) (13,14). The β-APP mRNA in the PC-12 cultures were further characterized as to isoform class. We found β-APP 751/770 mRNAs collectively were more abundant than β-APP 695 mRNA in untreated, bFGF-, and NGF-treated cells (data not shown). In addition, we did not observe a significant modulation of either class of β-APP isoform after hormone treatment. The minimal effects of NGF on β-APP mRNA transcription in PC-12 cells have been observed by Wion et al. (21) and Mobley et al. (22) but FGF effects have not been previously characterized.



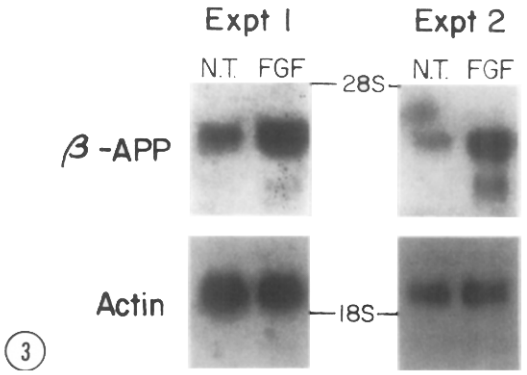


Figure 2 Northern blot analysis of bFGF treatment of PC-12 cells on β-APP expression. Equal amounts of RNA isolated from untreated (NT), bFGF-treated (FGF), and NGF-treated (NGF) cells were fractionated and hybridized with a β-APP probe (top panels), with a β-actin probe (middle panels), or with a c-fos probe (bottom panel). Two independent experiments (Expt 1 and 2) are presented in the left and right set of panels, respectively. The positions of 28S and 18S ribosomal RNAs are indicated.

Figure 3 Northern blot analysis of bFGF treatment of C6 cells on β-APP expression. Equal amounts of RNA isolated from untreated (NT) and bFGF-treated (FGF) cells were fractionated and hybridized with a β-APP probe (top panels) or a β-actin probe (bottom panels). Two independent experiments (Expt 1 and 2) are shown in the left and right panels, respectively. The positions of 28S and 18S ribosomal RNAs are indicated.

Since many cell types, including those of glial origin, respond to bFGF, we investigated the influence of this factor on β-APP expression in C6 cultured glial cells. Sub-confluent cultures of C6 cells were treated with 10 ng/ml (~0.5 nM) bFGF for 48 hours. No overt morphological changes were apparent prior to harvesting the cells for RNA extraction. Northern blots were prepared and hybridized with β-APP cDNA probe. C6 cultures treated with bFGF resulted in an induction of β-APP 3.6 kb and 2.0 kb mRNAs compared to untreated cultures (Figure 3). Actin mRNA levels were unchanged after bFGF application. In several different experiments, the induction of β-APP mRNA levels in C6 cells ranged from five- to ten-fold and was exclusively the β-APP

isoforms containing the Kunitz proteinase inhibitor (data not shown).

The stimulation of β-APP mRNA by bFGF in C6 astrocytoma cells has direct relevance to Alzheimer's disease (AD). Gliosis and β-amyloid deposition are classic features of AD (11,23,24). FGF, released upon neural injury, may promote both glial proliferation and excess β-APP levels, in particular, inhibitor containing isoforms. An increased, long-lasting elevation of β-APP immunoreactivity in reactive astrocytes following neuronal damage in adult rat brain has been reported (25) and it seems probable that bFGF is one of the injury-related factors responsible for β-APP induction. Elevated expression of β-APP by bFGF may contribute to amyloid formation, thereby compromising its potential neurotrophic effects observed for basal forebrain cholinergic neurons (26).

ACKNOWLEDGMENTS

We would like to acknowledge the Cal Bio Tissue Culture and DNA Synthesis Facitities and Eric Stoelting for art work. This research was supported in part by Daiichi Pharmaceutical Co.

REFERENCES

1. Gospodarowicz, D. (1988) In Biology of Growth Factors V (J.E. Kudow, D.H. MacLennen, A. Bernstein, A.I. Gotlieb, Eds.) 234, pp.23-39.

2. Barde, Y.-A. (1989) Neuron 2, 1525-1534.

3. Hefti, F., Hartikka, J., and Knusel, B. (1989) Neurobiol. Aging. 10, 515-533.

4. Togari, A., Baker, D., Dickens, G., and Gurdoff, G. (1983) Biochem. Biophys. Res. Comm. 114, 1189-1193.

5. Rydel, R.E. and Greene, L.A. (1987) J. Neurosci. 7, 3639-3653.

6. Neufeld, G, Gospodarowicz, D., Dodge, L. and Fujii, D.K. (1987) J. Cell. Physiol. 131, 131-140.

7. Edgar, D. and Thoenen, H. (1978) Brain Res. 154, 186-190.

8. Hatten, M.E., Lynch, M., Rydel, R.E., Sanchez, J., Joseph-Silverstein, J., Moscatelli, D., and Rifkin, D. (1988) Dev. Biol. 125, 280-289.

9. Rappolee, D.A., Mark, D., Banda, M.J. and Werb, Z. (1988) Science 241, 708-712.

10. Finklestein, S.P., Apostolides, P.J., Caday, C.G., Prosser, J., Philips, M.F. and Klagsburn, M. (1988) Brain Res. 253, 253-259.

11. Glenner, G. (1988) Cell 52, 307-308.

12. Kang, J., Lemaire, H.G., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K.H., Multhaup, G. and Beyreuther K. (1987) Nature 326, 733-736.

13. Ponte, P., Gonzalez-DeWhitt, P., Schilling, J., Miller, J., Hsu, D., Greenberg, B., Davis, K., Wallace, W., Lieberburg, I., Fuller, F. and Cordell, B. (1988) Nature 331, 525-527.

14. Tanzi, R., McClatchey, A.I., Lamperti, E.D., Villa-Komaroff L., Gusella, J.F. and Neve, R.L. (1988) Nature 331, 527-530.

15. Kitaguchi, N., Takahashi, Y., Tokushima, Y., Shiojiri, S. and Ito, H. (1988) Nature 331, 530-532.

16. de Sauvage, F. and Octave, J.-N. (1989) Science 245, 651-653.

17. Selkoe, D. (1989) Trends in Neurosci. 10, 181.

18. Greene, L.A. and Tischler, A.S. (1982) Adv. Cell. Neurobiol. 3, 373-414.

19. Chomczynski, P. and Sacchi, N. (1987) Analyt. Biochem. 162, 156-159.

20. Greenberg, M.E., Greene, L.A., Ziff, E.B. (1985) J. Biol. Chem. 260, 14101-14110.

21. Wion, D., Le Bert, M. and Brachet, P. (1988) Int. J. Devl. Neurosci. 6, 387-393.

22. Mobley, W.C., Valletta, J., Barrall, T., Long, J., Longo, F.M., Price, D. and Neve, R. (1989) Neurosci Mtg. Abst. 379.2

23. Duffy, P.E., Rapport, M. and Graf, L. (1980) Neurol. 30, 778-782.

24. Schechter, R., Yen, S.-H. C. and Terry, R.D. (1981) J. Neuropathol. Exp. Neurol. 40, 95-101.

25. Siman, R., Card, P., Nelson, R.B. and Davis, L.G. (1989) Neuron 3, 275-285.

26. Anderson, K.J., Dam, D., Lee, S. and Cotman, C. (1988) Nature 332, 360-361.