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Basic fibroblast growth factor protects striatal neurons in vitro from NMDA-receptor mediated excitotoxicity

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Basic fibroblast growth factor (bFGF) promotes the survival and outgrowth of neurons. In this study the neuroprotective effects of bFGF were examined in 12–18-day-old cultured striatal neurons exposed to glutamic acid, kainic acid (KA), and quinolinic acid (QA), an *N*-methyl-D-aspartate (NMDA)-receptor agonist. Results showed that preincubation with bFGF (6 pM) from the day of plating significantly increased the survival of striatal neurons treated for 3 h with glutamate (3 mM) or QA (1 mM), but had little effect on KA (1 mM) induced toxicity. Moreover, maximum protection by bFGF against glutamate neurotoxicity was observed in cultures treated as little as 2 h before glutamate exposure. These results show that bFGF markedly protects striatal neurons from NMDA-receptor induced neurotoxicity.

Fibroblast growth factors (FGFs) are a family of heparin binding proteins that have multiple biological activities in mammalian cells[6,10,11,22]. Two forms of FGF, acidic and basic, are highly concentrated in the adult central nervous system and are known to markedly enhance the survival and growth of glia and neurons in vitro[15,17,21,23,24]. Basic FGF has more widespread and more potent neurotrophic effects than aFGF[23] and has been found to improve the survival of injured neurons in vivo[2,18].

Basic FGF has also been found to significantly increase the survival of hippocampal neurons exposed to glutamic acid induced toxicity[15]. Since neostriatal neurons are also highly vulnerable to the toxic effects of excitatory amino acids[8,12], and are among those cells whose survival and growth in culture is markedly enhanced by bFGF[23,25], we undertook to determine in this study whether bFGF also protects cultured striatal neurons when they are exposed to glutamate and the glutamate receptor agonists quinolinic acid (QA) and kainic acid (KA). Results show that treatment with bFGF can significantly protect striatal neurons from the toxicity caused by glutamate and QA, an agonist of the *N*-methyl-D-aspartate (NMDA) receptor.

Striatal tissue from postnatal day 0 (newborn) rat pups (Sprague–Dawley) was prepared for culture as previously described[8]. Cells, including both neurons and glia, were plated (10^6 per dish) onto polylysine-coated coverslips in culture dishes containing 1.5 ml of media (50% DMEM, 50% HAMS-F12, 10% heat-inactivated horse serum, supplemented with 100 units/ml of penicillin/streptomycin and 4 g/l of glucose), and incubated at 37°C, in 5% CO_2. Basic FGF (6 pM; Peprotech, Inc., Rocky Hill, NJ) was added to some culture dishes on the day of plating and in subsequent feedings. Media were replaced every 3–4 days and cytosine arabinoside (40 μM), an inhibitor of mitosis, was added on the 5th day to prevent overgrowth by glial cells. Under phase contrast microscopy (32 × objective lens), selected fields containing approximately 20–30 neurons were photographed with Polaroid 665 film before and after exposure to different excitatory amino acids and neuron survival was quantitated in the photographs. Cavitation and shrinking of the cell body, as well as beading and/or disappearance of processes were required as criteria for cell death. Confirmation of the neuronal elements in culture has already been described in detail elsewhere[9].

Experiments were conducted in 12- to 18-day-old cultures since our previous studies had shown that electrophysiological responses to glutamate and NMDA agonists, and vulnerability to their toxicity were maximal in older cultures[8,13]. Striatal neurons treated with bFGF (6 pM from the day of plating) and exposed to glutamate (1 mM) for 3 h (Fig. 1), exhibited significantly more

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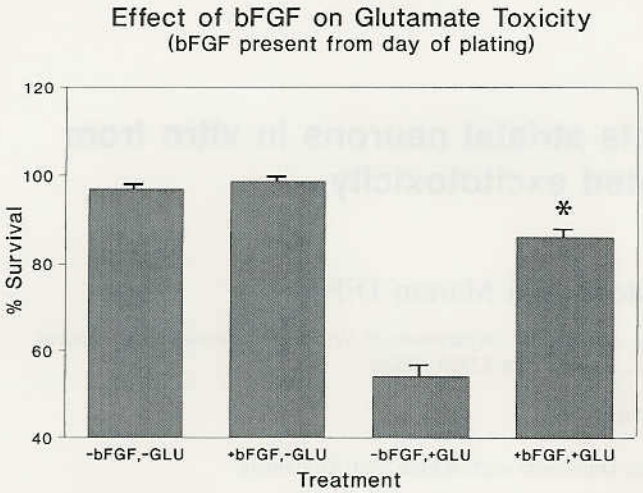

Fig. 1. Survival of 12–18-day-old striatal cultures treated with or without bFGF (6 pM from day of plating) and glutamate (GLU; 1 mM). For each treatment condition the values represent the mean (± S.E.M.) derived from all culture dishes (12–22 dishes per condition) obtained from 5 to 7 experiments. Glutamate (3 mM) was added for 3 h, and the survival of neurons was calculated. Survival of neurons treated with bFGF and glutamate (+bFGF, + GLU) was significantly increased (one-way ANOVA: $F_{3,69} = 86.2$, $P < 0.001$; post hoc t-test at $^*P < 0.001$) compared to those neurons not treated with bFGF and exposed to glutamate (–bFGF, + GLU). Survival was not affected in control cultures that were not exposed to glutamate (–bFGF, –GLU; +bFGF, –GLU.

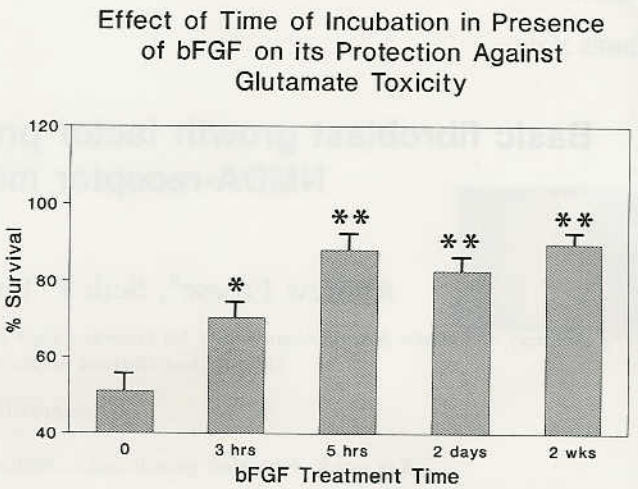

Fig. 2. The effect of incubation of 14-day-old striatal cultures with 6 pM bFGF for various treatment periods (0 h to 2 wks) on the survival of striatal neurons exposed to 1 mM glutamate (GLU) for 3 h. Values represent the mean percent survival in 7–12 dishes per condition from 3 experiments. Survival of neurons exposed to glutamate was significantly increased in all cultures incubated with bFGF (one-way ANOVA: $F_{4,39} = 16.7$, $P < 0.001$; post hoc t-test at $^*P < 0.01$ and $^{**}P < 0.001$). In the absence of bFGF (0 condition), mean survival after glutamate was 51.1 ± 4.7%. (Results not shown: in the absence of both bFGF and glutamate mean percent survival was 97.2 ± 2.6% and for neurons exposed to bFGF for any length of time without glutamate mean survival was 98.6 ± 1.3%.)

survival ($\bar{x}$ = 86.1% survival ± 2.2) than those neurons treated with glutamate alone ($\bar{x}$ = 54.1% survival ± 3.1). Neuron survival in response to glutamate in 14-day-old cultures (Fig. 2) was significantly improved with bFGF pretreatment periods of 2 weeks ($\bar{x}$ = 89.7% survival ± 3.1 and 2 days ($\bar{x}$ = 82.7% survival ± 4.0). Protective effects of bFGF were maximal even when an incubation period as brief as 2 h preceded glutamate exposure (total bFGF treatment time: 5 h, $\bar{x}$ = 88.1% survival ± 4.5). A smaller but significant protective effect was achieved when bFGF treatment coincided (bFGF treatment: 3 h) with the period of glutamate incubation ($\bar{x}$ = 70.4% ± 4.4 with bFGF vs. $\bar{x}$ = 51.1 ± 4.7 without bFGF). Since our previous work showed that the toxic effects of glutamate on striatal cultures were largely mediated at the NMDA receptor[8,13], we also treated cultures with QA (Fig. 3). The survival of neurons exposed to QA (1 mM) was also significantly improved in cultures incubated with bFGF ($\bar{x}$ = 96.3% survival ± 1.6) compared to cultures without bFGF ($\bar{x}$ = 77% ± 4.0) (Fig. 4). In contrast, the survival of neurons exposed to 1 mM KA was not significantly improved by preincubation with bFGF (Fig. 5, $\bar{x}$ = 73.5% survival ± 7.1 without bFGF and $\bar{x}$ = 69.2% survival ± 4.1 with bFGF).

The main findings in this study are that bFGF partially protects striatal neurons from glutamate-induced excitotoxicity by attenuating NMDA-receptor-mediated toxicity and not KA-receptor-induced injury. The mechanism by which bFGF lessens excitotoxic effects is not completely clear but may be related to the regulation of intracellular Ca^{2+}. Mattson et al.[15,16] found that bFGF protected hippocampal neurons in culture from glutamate by causing a reduction in the glutamate induced elevation of intracellular Ca^{2+}, which is thought to initiate the sequence of intracellular events leading to cell death. Since the glutamate-induced rise in intracellular Ca^{2+} is mediated primarily through NMDA receptors[14, 20], our finding that bFGF was most affective in reducing NMDA-receptor-mediated toxicity in striatal cultures is not surprising. Similar to hippocampal cultures[15], maximum protection by bFGF in striatal cultures required several hours of incubation with the growth factor prior to glutamate exposure. There is evidence that ongoing mRNA and protein synthesis during the pretreatment period with bFGF is necessary to diminish the toxic effects caused by glutamate[15].

Results of this study raise speculation that a dysfunction in bFGF could be involved in the neuropathology

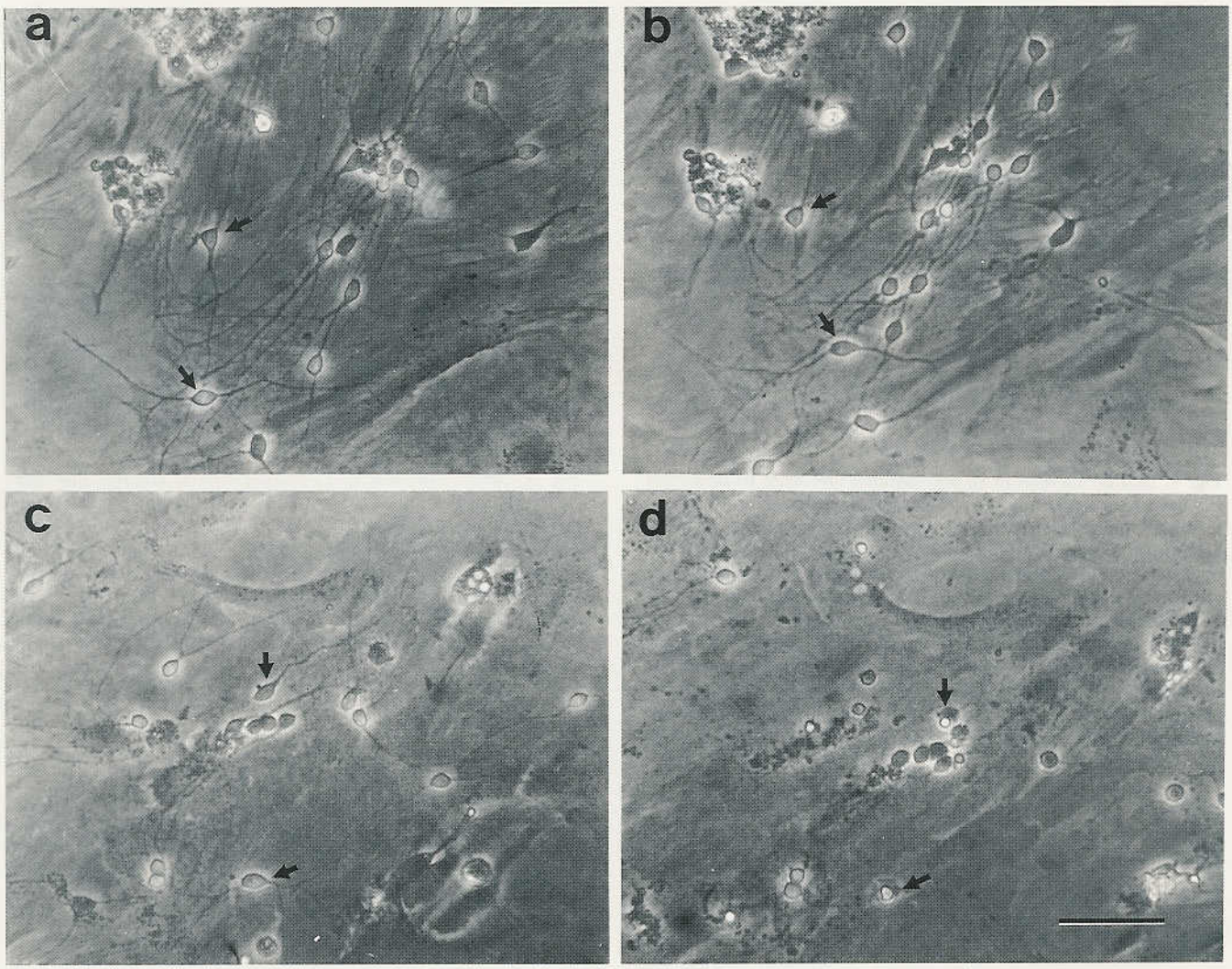

Fig. 3. Striatal cells cultured for 14 days with (a,b) or without bFGF (c,d): a and c are two different fields that were photographed before exposure to 1 mM QA. b and d are the same fields shown in a and c, respectively, photographed after the neurons were incubated for 3 h with 1 mM QA. Neurons treated with bFGF (a,c) show little change after QA exposure whereas those not treated with bFGF (c,d) and exposed to QA show a marked loss of processes and shrinkage and vacuolization of cell bodies. Compare corresponding arrows in a and b and in c and d. Bar = 50 μm.

of Huntington's disease, where excitotoxicity is thought to contribute to the severe loss of striatal projection neurons[4]. In addition to the present findings, other evidence exists to support a neurotrophic role for the growth factor in the caudate-putamen. For example, when introduced into the striatum in gelfoam implants, bFGF has been shown to reverse the degeneration of nigrostriatal dopaminergic axons caused by 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine (MPTP) lesions in the substantia nigra[18]. Moreover, in striatal cultures, bFGF treatment significantly increases the density and neuritic outgrowth of neurons that contain γ-aminobutyric (GABA)[25], the neurotransmitter found in striatal projection neurons. The trophic effects of bFGF on neuritic outgrowth are not mediated by glial cells and are not found when GABAergic neurons are treated with nerve growth factor under the same culturing conditions.

Both bFGF and its receptor protein have been localized to adult neostriatal neurons by immunohistochemistry[19] (Zhou and DiFiglia, unpublished observations). However, the mechanism by which bFGF is released from cells is still unclear, because the polypeptide lacks a signal peptide sequence needed for transport to the endoplasmic reticulum where proteins destined for secretion are directed during synthesis[1,5]. Interestingly, its recent localization to multiple subcellular compartments in hepatoma

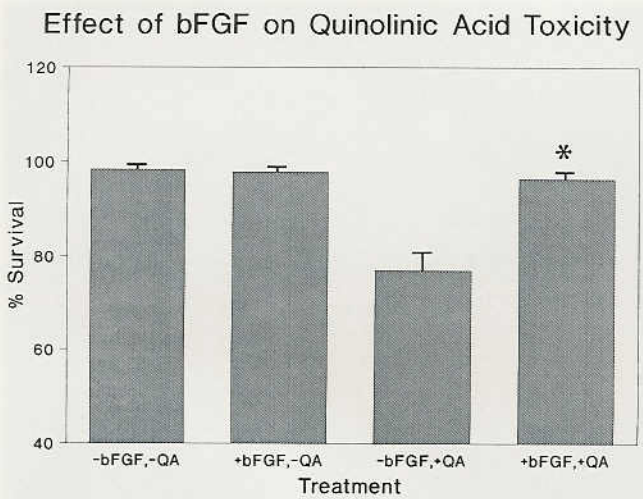

Fig. 4. Neuron survival in striatal cultures that were treated from the day of plating with 6 pM bFGF or without bFGF and exposed to 1 mM quinolinic acid (QA) for 3 h or not treated with the excitotoxin. For each condition values represent the mean ± S.E.M. of results of 11–12 dishes per condition from 4 experiments. Survival of neurons treated with QA was significantly improved by bFGF treatment (one-way ANOVA: $F_{3,43}$, = 19.2, $P < 0.001$; post hoc t-test at $^*P < 0.001$). Survival was unaffected in control cultures not exposed to bFGF or QA. Thus, the difference between survival of cells exposed to both bFGF and QA and survival of cells exposed to only bFGF or to nothing at all was not statistically significant, indicating complete protection of bFGF against QA-induced toxicity.

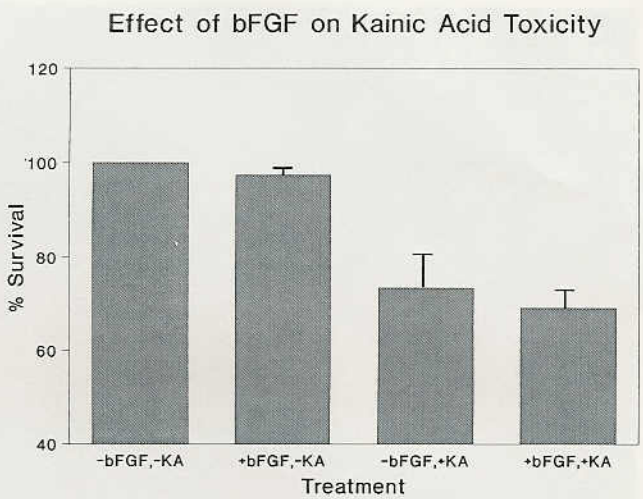

Fig. 5. Neuron survival in 12-day-old striatal cultures that were treated with or without 6 pM bFGF from the day of plating and incubated with or without 1 mM KA for 3 h. Survival of neurons exposed to both bFGF and KA (+FGF, +KA) was not significantly improved compared to those treated with KA alone (one-way ANOVA: $F_{3,15}$ = 18.45, $P < 0.001$, post hoc t-test for –bFGF, +KA vs. +bFGF, +KA, not significant). Survival was unaffected in control cultures not exposed to KA and treated with or without bFGF.

cells[3] and in striatal neurons[19] suggests that bFGF could have both secretory and intracellular functions.

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