

Acidic Fibroblast Growth Factor Infusion Reduces Ischemic CA1 Hippocampal Damage in the Gerbil

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ABSTRACT: Occlusion of the carotid arteries for 5 minutes in the Mongolian gerbil results in selective necrosis of CA1 pyramidal neurons. In the present experiments we studied whether intraventricular infusion of acidic fibroblast growth factor (aFGF) could attenuate this damage. Intraventricular infusions of bovine serum albumin (BSA-10 ng/h) or aFGF (1, 10 or 100 ng/h) were started 2 days prior to 5 minutes of bilateral carotid occlusion and continued for 5 days post-ischemia. The brains were perfused and fixed at 5 days post-ischemia and histological assessment of CA1 damage was made. Animals receiving intraventricular infusions of 10 or 100 ng/h aFGF showed a significant reduction of CA1 neuronal damage in comparison to no treatment ischemic controls (no treatment - 8 ± 1 ; aFGF 10 ng/h - 147 ± 28 ; aFGF 100 ng/h - 168 ± 35 cells/mm CA1; $P < 0.05$ for both aFGF groups). The results indicate that aFGF infusion can attenuate the severity of ischemic neuronal necrosis in the gerbil hippocampus.

RÉSUMÉ: L'infusion du facteur de croissance acide des fibroblastes diminue le dommage ischémique aux neurones de la zone CA1 de l'hippocampe chez la gerboise. L'occlusion des carotides pendant 5 minutes chez la gerboise mongole produit une nécrose sélective des neurones pyramidaux CA1. La présente étude vise à déterminer si une infusion intraventriculaire de facteur de croissance acide des fibroblastes (aFGF) pouvait atténuer ce dommage. Des infusions intraventriculaires d'albumine sérique bovine (BSA-10 ng/h) ou d'aFGF (1, 10 ou 100 ng/h) ont été commencées 2 jours avant une occlusion bilatérale des carotides d'une durée de 5 minutes et continuées pendant 5 jours post-ischémie. Les cerveaux étaient perfusés et fixés au jour 5 post-ischémie et on procédait à une évaluation histologique des dommages subis par les neurones CA1. Les animaux qui recevaient des infusions intraventriculaires d'aFGF à 10 ou 100 ng/h présentaient une diminution significative des dommages subis par les neurones CA1 par rapport aux contrôles ischémiés mais non-traités (contrôles - 8 ± 1 ; aFGF10 ng/h - 147 ± 28 ; aFGF 100 ng/h - 168 ± 35 cellules/mm CA1; $p < 0.05$ pour les deux groupes aFGF). Ces résultats indiquent qu'une infusion d'aFGF peut atténuer la sévérité de la nécrose neuronale ischémique dans l'hippocampe de la gerboise.

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A recent study has indicated that the neurotrophic factor, fibroblast growth factor (FGF) reduces the killing of cultured hippocampal neurons by glutamate.¹ In addition, these studies indicated that alterations of intracellular calcium may be a common factor for both the damaging actions of glutamate and the protective actions of FGF. Since increases of extracellular glutamate and intracellular calcium are proposed to be important pathogenetic factors in the process of selective neuronal damage in ischemia²⁻⁴ we investigated whether intraventricular infusions of FGF might protect vulnerable hippocampal neurons from ischemia.

MATERIALS AND METHODS

The experiments were performed on adult male Mongolian gerbils (50 - 70 g; Tumblebrook Farms, West Brookfield, MA)

that were allowed free access to food and water. Two days prior to ischemia the animals were anesthetized with intraperitoneal pentobarbital (50 mg/kg) and an ALZET osmotic pump (model 1007D, ALZA Corp., Palo Alto, Ca), connected to a polyethylene tube, was placed in the subcutaneous tissue of the back. The free end of the polyethylene tube was tunneled subcutaneously to the skull where it was attached to a 27 gauge needle cannula which was placed in the right lateral ventricle (0.2 mm posterior to the bregma, 1.2 mm lateral to the sagittal suture and 1.7 mm deep). The cannula was secured to the skull by the use of cyanoacrylate glue. The osmotic pump and tube were filled with sterile artificial CSF (NaCl 120 mM; NaHCO₃ 25 mM; KCl 3.3 mM; CaCl₂ 2.6 mM; NaH₂PO₄ 1.23 mM; MgSO₄·7H₂O 1.2 mM; glucose 10 mM) containing either recombinant acidic fibroblast growth factor⁵ (aFGF; 2, 20 or 200 mg/ml; gift of Dr.

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M. Jaye, Rorer Biotechnology Inc., King of Prussia, PA), or bovine serum albumin (BSA; 20 mg/ml; Sigma Chemical Corp., St. Louis, MO) and allowed to equilibrate in a dish of artificial CSF for 4 h prior to subcutaneous insertion. The mean pumping rate of the infusion units was $0.55 \pm 0.02 \mu\text{l/h}$ (data provided by ALZA Corp, Palo Alto, CA) which gave delivery rates of about 1, 10 and 100 ng/h for the aFGF infused animals and of 10 ng/h for the BSA infused animals. Controls consisted of non-ischemic and ischemic gerbils which received no intraventricular infusions.

Two days after placement of the osmotic pumps the animals were anesthetized with 2.5% halothane in 30% O₂ - 70% N₂O and the carotid arteries were surgically exposed via a midline cervical incision. Both carotid arteries were occluded with small arterial clips and the halothane-anesthetic gas mixture was discontinued. At the end of 5 min occlusion, the arterial clips were released, the return of carotid blood flow visually confirmed and the neck wound was closed with metal skin clips. Body temperature was monitored rectally and maintained at 37-38°C during the occlusion by use of a heating pad and incandescent lamp.

Five days after carotid occlusion the animals were anesthetized with i.p. pentobarbital and the brain was fixed *in situ* via intracardiac perfusion of 20 ml 0.9% saline, followed by 250 ml 10% formaldehyde. The fixed brain was subsequently removed from the skull, placed in 10% formaldehyde and processed for paraffin sections and cresyl violet staining.⁶ The extent of hippocampal damage was evaluated by counting the intact neurons of the CA1 region of the right hippocampus in

sections taken at approximately -1.4, -1.7 and -2.0 mm to the bregma.⁷ The CA1 length was measured with a video program and results are expressed as cells per 1 mm length CA1. Differences in mean cell densities were analyzed using Wilcoxon's ranked sum test for unpaired samples with $p < 0.05$ being needed for statistical significance.

RESULTS

Intraventricular cannulation, infusion and ischemia were generally well tolerated by the experimental animals. Thus of a total of 26 exposed animals, 5 deaths were recorded. Three of these deaths were in the 1 ng/h aFGF group and showed the pattern of acute seizures with pulmonary edema coming on immediately after release of the carotid clips. One animal in each of the 10 and 100 ng/h group showed onset of seizures with pulmonary edema at 0.5 and 2 h post carotid occlusion, respectively.

The mean rectal temperature during ischemia was 37.7 ± 0.1 , 37.3 ± 0.2 , 37.0 ± 0.2 , 37.4 ± 0.1 , and $37.6 \pm 0.3^\circ\text{C}$ for the ischemic control, 10 ng/h BSA, 1, 10 and 100 ng/h aFGF groups, respectively. Body weight of all gerbils was between 45-63 g.

Table 1 gives the values for intact CA1 neurons in the various experimental groups and shows that aFGF infusion at 10 and 100 ng/h had a significant protective effect on ischemic CA1, whereas 1 ng/h was without protective effect. Examination of the CA1 of the 10 and 100 ng/h groups indicated that the distribution of surviving neurons was homogenous and not localized to specific areas of the CA1 (see Figure 1).

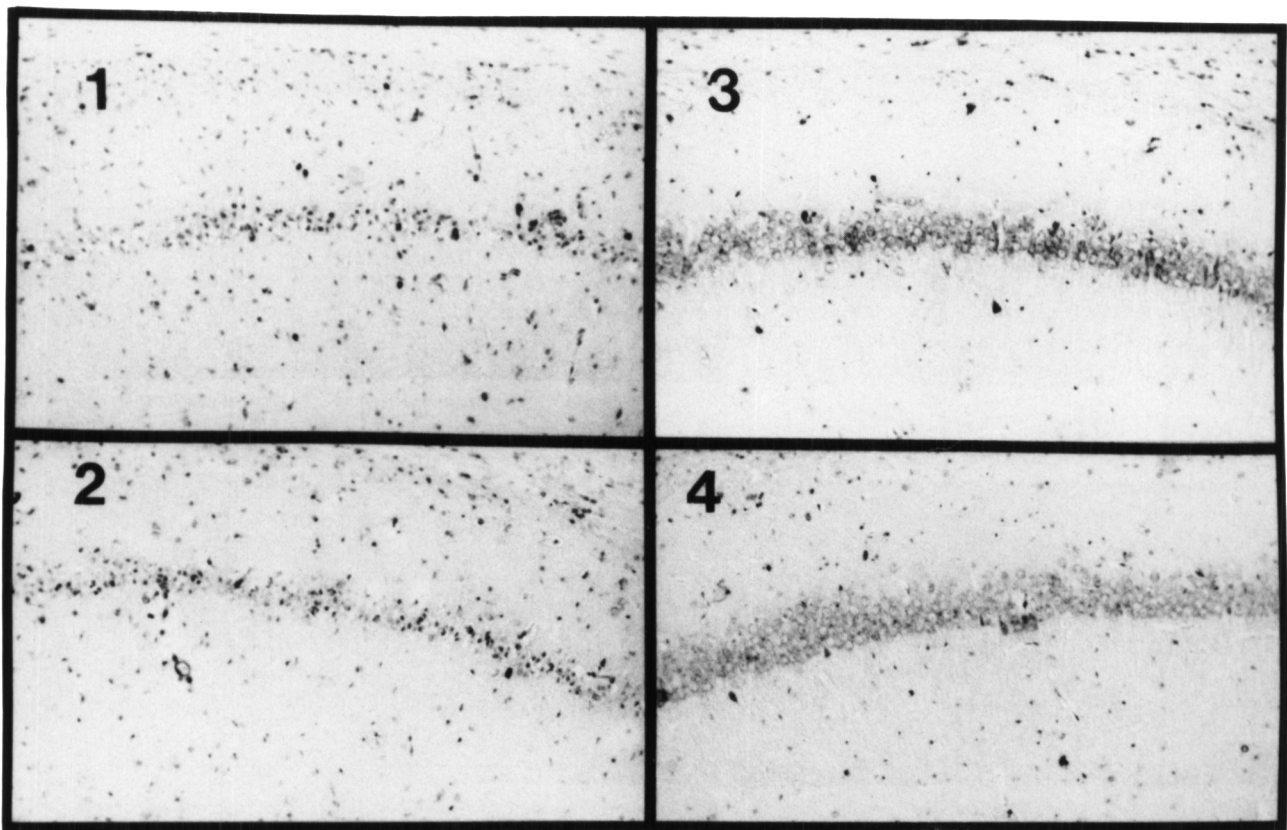


Figure 1 — Microscopic appearance of CA1 section of hippocampus of ischemic gerbils pretreated with intraventricular infusions of 1. BSA 10 ng/h, 2. aFGF 1 ng/h, 3. aFGF 10 ng/h or 4. aFGF 100 ng/h.

Table 1. Hippocampal CA1 neuronal density in post-ischemic gerbils receiving intraventricular infusions of BSA or aFGF

Experimental Group	n	cells/mm CA1
1. Non-ischemia	8	197 ± 5
2. Ischemic - no treatment	7	8 ± 1
3. Ischemic - BSA	6	18 + 9
4. Ischemia - aFGF		
1 ng/h	2	4 + 2
10 ng/h	9	147 + 28*
100 ng/h	4	168 + 35*

Values are means + SEM. n = number of animals.

* $p < 0.05$, BSA or aFGF groups compared to no treatment ischemic control.

DISCUSSION

The results of the present study confirm and extend observations that have indicated that neurotrophic factors have an ability to protect the central nervous system in a variety of damaging situations. These include the demonstrations that intraventricular infusions of nerve growth factor⁸⁻¹⁰ or basic FGF¹¹ prevent death of basal forebrain cholinergic neurons after fimbria-fornix transections, that repeated intracisternal injections of basic FGF (bFGF) prevent thalamic degeneration after cortical infarction in the rat,¹² and that local injection of aFGF protects the nigrostriatal dopaminergic system from the toxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.¹³ In addition, Oomura et al.¹⁴ have reported in abstract format that intra-ventricular infusion of aFGF attenuates ischemic CA1 hippocampal damage in the gerbil; an observation that directly supports the present results.

Fibroblast growth factors have been shown to have prominent mitogenic actions on fibroblasts, endothelial cells and other cells of mesenchymal origin.¹⁵ In addition, aFGF and bFGF promote survival and proliferation of CNS glial cells and a wide variety of CNS neurons *in vitro*.¹⁶ A possible role in the reaction to and repair of the central nervous system to injury has been proposed by Finklestein et al.,^{17,18} who have shown prominent increases in both bFGF and aFGF in tissue surrounding focal cortical lesions.

Although the mechanism(s) by which FGF's promote the survival of neural tissue after injury is as yet unknown, the studies of Mattson et al.,¹ showing that pretreatment with RNA or protein synthesis inhibitors prevents the protective action of FGF in glutamate-treated hippocampal neuron cultures, indicate a possible role for FGF in the induction or regulation of a RNA or protein product which influences hippocampal neuronal survival. Since other studies have indicated that inhibition of protein synthesis reduces CA1 neuronal death in ischemia,¹⁹ the *in vitro* data of Mattson et al.¹ seem to indicate that the glutamate neurotoxicity sparing action of FGF is mediated via a protein/RNA system that is different from that believed responsible for *in vivo* ischemic cell death.¹⁹ Further experimental studies will be needed to establish these various possibilities.

Reduction of ischemic brain temperature has been shown to be a major protective mechanism for a variety of pharmacological "anti-ischemic" agents.^{20,21} Although the intraventricular cannulation and process of infusion can be eliminated as causes for such temperature variations (i.e.; no protective effect in BSA

infused group), it remains remotely possible that aFGF itself could act to enhance intra- or post-ischemic brain temperature reduction. In addition, the increased rate of acute post-ischemic death in the aFGF-infused animals may indicate that aFGF sensitizes the animals to the acute effects of cerebral ischemia. As described, these deaths were characterized by seizures with pulmonary edema which came on at or shortly after the termination of ischemia. The above questions remain the topics of ongoing investigation.

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