BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1998 Nov; 19 (6): 527 - 530

Basic fibroblast growth factor protected forebrain against ischemia-reperfusion damage in rats

LIU Xuan, ZHU Xing-Zu[1] (*Department of Pharmacology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China*)

KEY WORDS basic fibroblast growth factor; reperfusion injury; visual cortex; dopamine; water; sodium; potassium; high pressure liquid chromatography; corpus striatum

AIM: To study the effect of basic fibroblast growth factor (bFGF) on acute forebrain ischemia-reperfusion injury in rats. **METHODS**: Both vertebral arteries were occluded by electrocautery and severe but transient bilateral cerebral ischemia was produced by clamping both common carotid arteries for 20 min in rats. The contents of dopamine (DA) and 3, 4-dihydroxyphenylacetic acid (DOPAC) in striatum were determined by HPLC with electrochemic detector (HPLC-EC) at 3, 6, 12, 24 h after the onset of reperfusion. The contents of sodium, potassium in forebrain were determined by atomic absorption spectro-photometric method at 6-h reperfusion. Morphological changes of the striatum were also examined. **RESULTS**: At 6-h reperfusion, the DA content in striatum decreased from (99 ± 16) $\mu g \cdot g^{-1}$ (protein) in sham-operation group to (70 ± 20) $\mu g \cdot g^{-1}$ (protein); the water and the sodium contents in forebrain increased from 77.34 % ± 0.19 % to 79.6 % ± 0.6 % and from (9.3 ± 0.6) to (10.5 ± 0.6) $mg \cdot g^{-1}$ (dry weight), respectively. bFGF (iv 45 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for 3 h) from the start of reperfusion prevented the decrease of DA in striatum and the increases of water and sodium contents in forebrain. Histological examination also indicated that bFGF ameliorated the injury of neurons. **CONCLUSION**: bFGF prevented the brain from injury of ischemia and reperfusion.

Basic fibroblast growth factor (bFGF) is a 18-kDa polypeptide with potent survival-promoting and protecting effects on central nervous system (CNS) neurons[1-3]. bFGF protected cultured neurons against a number of toxins such as anoxia, hypoglycemia, excitatory amino acids and oxygen free radicals[4]. These effects appears to be mediated through signal transduction cascades initiated by binding of bFGF to its high affinity cell-associated receptor, resulting in new cellular gene expression and protein synthesis[5]. The therapeutic efficacy of bFGF *in vivo* has also been demonstrated by a number of laboratories through central administration of bFGF in rodent models of ischemia[6,7]. In gerbils, central administration of bFGF decreased brain injury associated with transient forebrain ischemia[8]. bFGF is also a potent vasodilator besides its direct cytoprotective effects. It increased regional cerebral blood flow in rat and rabbit brains[9]. Thus, the effect of bFGF *in vivo* may include direct cytoprotection as well as effects on regional cerebral blood flow. The potential effect of systemically administered bFGF was evaluated in cats[10] and rats[11,12]. Although bFGF may not be expected to cross the blood-brain-barrier (BBB) under normal circumstances, the damaged BBB in ischemia could allow bFGF to penetrate ischemic brain tissue[12]. However, these reports only tested the effect of bFGF on injury resulted from focal cerebral ischemia using middle cerebral artery (MCA) occlusion models. The effect of systemical infusion of bFGF on injury following temporary forebrain ischemia was tested in this paper.

MATERIALS AND METHODS

Chemicals Recombinant human bFGF was provided by Torita Bio-Pharma Co Ltd (Zhuhai, China). DA and DOPAC were purchased from Sigma Chemical Co (St Louis MO, USA).

Rats Wistar rats (♀, $n = 74$), weighing 200 - 240 g, were obtained from the Shanghai Experimental Animal Center of Chinese Academy of Sciences (Grade Ⅱ, Certificate № 005). Rats were randomized into 6 groups. Group A:

[1] Correspondence to Prof ZHU Xin-Zu. Phn 86-21-64311-833, ext 309. Fax 86-21-6437-0269. E-mail xzzhu@server.shcnc.ac.cn
Received 1998-01-20 Accepted 1998-06-18

sham operation. Group B to E (treated with saline): ischemia for 20 min and recirculation for 3, 6, 12, or 24 h, respectively. Group F and G (treated with bFGF from the start of reperfusion, iv 45 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for 3 h): ischemia for 20 min and recirculation for 6 or 12 h, respectively.

The 4-vessel occlusion method[13] Rats were anesthetized with chloral hydrate (400 $mg \cdot kg^{-1}$, ip). Bilateral vertebral arteries were occluded by electrocautery. After 2 h, bilateral cerebral ischemia was produced by clamping both common carotid arteries for 20 min, recirculation was started by removing the carotid clamps. The body temperature of rat was kept at 36.5 ℃ on a warm plate with a lamp in air-conditioned laboratory.

Histological examination At the desired timepoints, the rats were perfused transcardially with 4 % paraformaldehyde in phosphate-buffered saline (0.1 $mol \cdot L^{-1}$, pH 7.4). The brain tissues were processed for paraffin sections. Histological sections 4 μm were stained with hematoxylin and eosin.

Determination of DA and DOPAC contents[14] At the desired timepoint, the rats were decapitated and the right striatum were dissected on ice, weighed, and homogenized with 0.4 mL of ice-cold perchloric acid 0.1 $mol \cdot L^{-1}$. Homogenates were centrifuged at 17 000 $\times g$ for 15 min at 4 ℃. Supernatants were used for the measurement of DA and DOPAC and the pellets were used for protein assay (Method of Lowry *et al* 1951). Supernatant (20 μL) was injected into an HPLC system with electrochemic detector (BAS). The detection limit was 10 pg.

Determination of brain water, sodium, and potassium contents[13] The rat left brain hemisphere was dried at 100 ℃ for 12 h to determine the water content. Dry samples were digested in nitric acid. The sodium and potassium concentrations were analyzed with a Hitachi Z-8100 Polarized Zeeman atomic absorption spectrophotometer.

Statistics The results were expressed as $\bar{x} \pm s$, and assessed by 2-tailed t test.

RESULTS

At 6-h recirculation, the neurons in striatum of saline-treated group exhibited early damage such as nuclear pyknosis, but the neurons of bFGF-treated group were nearly normal. At 12-h recirculation, the edema of tissue and empty spaces appeared in the saline-treated group. However, the neurons of bFGF-treated group exhibited lower extent of damage (Fig 1).

The time profiles of DA and DOPAC contents in striatum after ischemia-reperfusion injury or sham-operation showed that DA level decreased at 6-h recirculation and remained at low level at 12-h as well as 24-h recirculation. The DOPAC content markedly increased at 3-h but decreased to normal level at 6-h, 12-h, and 24-h recirculation (Fig 2).

At 6-h recirculation, water and sodium contents in forebrain increased in saline-treated group besides the decrease of DA level in striatum. However, bFGF-treated group had markedly higher DA level and lower water and sodium contents comparing with saline-treated group. On the other hand, similar levels of DOPAC and potassium were found in all groups (Tab 1).

Tab 1. Effect of bFGF (45 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for 3 h) on DA and DOPAC levels in striatum; water, sodium and potassium contents in forebrain of rats at 6-h reperfusion. $n = 6$ rats. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* sham-operation group.

Group	Sham-operation	Saline-treated	bFGF-treated
DOPAC/$\mu g \cdot g^{-1}$ *	20 ± 4	19 ± 4	19 ± 3
DA/$\mu g \cdot g^{-1}$ *	99 ± 16	70 ± 20^b	102 ± 17
Water/%	77.3 ± 0.2	79.6 ± 0.6^c	77.1 ± 0.6
Sodium/$mg \cdot g^{-1}$ #	9.3 ± 0.6	10.5 ± 0.6^c	8.6 ± 0.5
Potassium/$mg \cdot g^{-1}$ #	17.9 ± 1.8	18.7 ± 1.3	17.8 ± 0.8

* means $\mu g \cdot g^{-1}$ (protein)
\# means $mg \cdot g^{-1}$ (dry brain)

DISCUSSION

Neurons in striatum are highly vulnerable to ischemic insult. Ischemia caused depletion of DA in this area[15]. In the present study, the histological experiment indicated that the neurons exhibited damage after ischemia-reperfusion injury. The time profiles of DA and DOPAC levels in the present study were consistent with previous reports[15]. The peak level of DOPAC at 3-h recirculation could be predominantly related to free DA in presynaptic nerve terminals

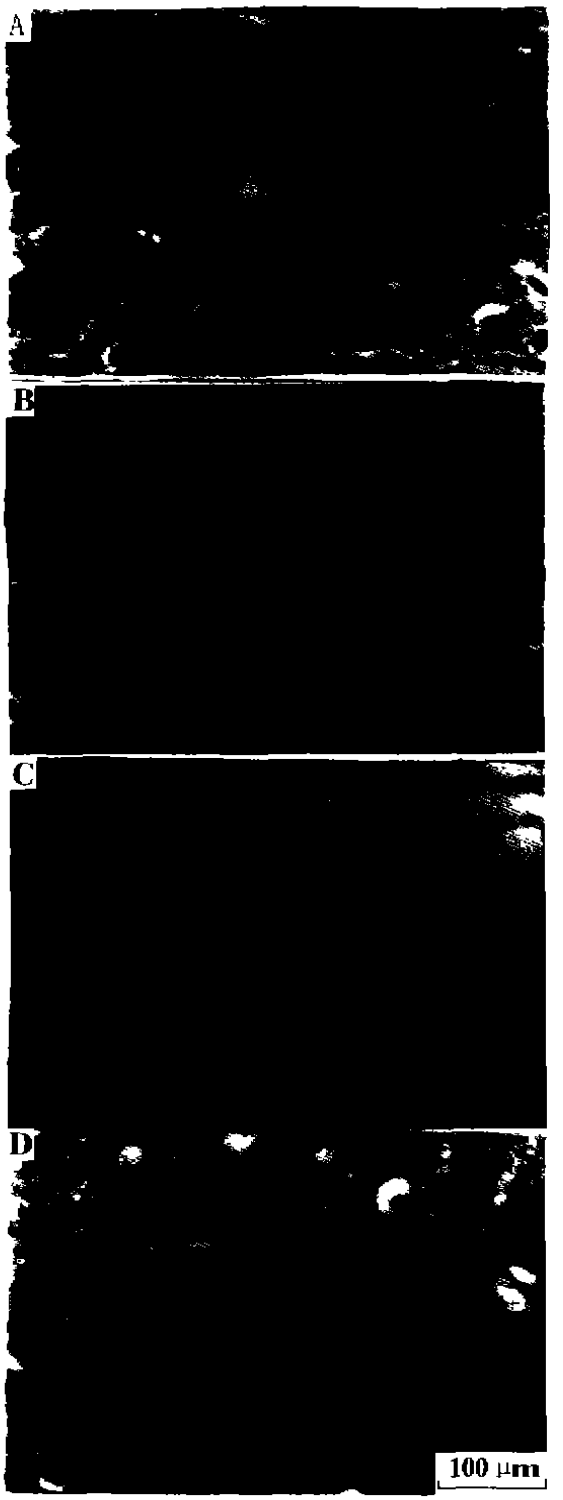

Fig 1. Effect of bFGF (45 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for 3 h, iv) on neuronal injury caused by ischemia-reperfusion insult. H&E stain × 132. A) 6-h recirculation, saline-treated; B) 6-h recirculation, bFGF-treated; C) 12-h recirculation, saline treated; D) 12-h recirculation, bFGF treated.

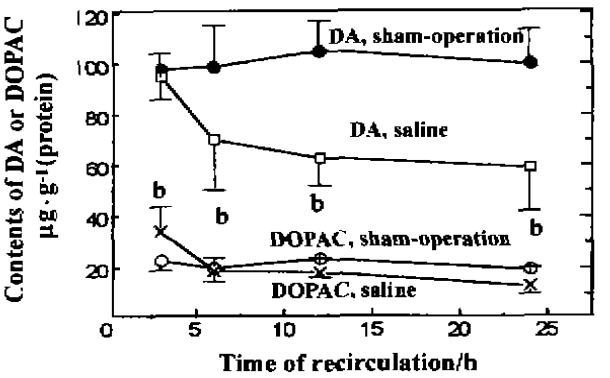

Fig 2. DOPAC and DA contents in striatum after ischemia-reperfusion or sham-operation. $n = 6$ rats. $\bar{x} \pm s$. $^bP < 0.05$ *vs* sham-operation group.

at earlier time periods. At 6-h recirculation as well as later timepoints, the DA depletion were apparent while the DOPAC content returned to nearly normal level. The depletion of DA was presumably the result of decreased blood supply and anoxia which produced changes in synthesis, release and re-uptake of DA in the ischemic areas. The continued release and reduced synthesis of DA may be the main causes.

Since the reduction of DA content became significant at 6-h recirculation, the protective effects of bFGF was tested at this timepoint. Intravenous infusion of bFGF was effective in ameliorating neuron damage and preventing the decrease of DA content in striatum and the increase of water and sodium contents in forebrain. Our results indicated that bFGF was able to protect the brain from damage induced by ischemia-reperfusion insult in 4-vessel occlusion model in rats.

REFERENCES

1 Walicke PA. Basic and acid fibroblast growth factors have trophic effects on neurons from multiple CNS regions. J Neurosci 1988; 8: 2618 – 27.

2 Louis JC, Magal E, Gerdes W, Seifert W. Survival-promoting and protein kinase C-regulating roles of basic FGF for hippocampal neurons exposed to phorbol ester, glutamate and ischemia-like conditions. Eur J Neurosci 1993; 5: 1610 – 21.

3 Maiese K, Boniece I, Demeo D, Wagner JA. Peptide growth factors protect against ischemia in culture by preventing nitric oxide toxicity. J Neurosci 1993; 13: 3034 – 40.

4 Freese A, Finklestein SP, DiFiglia M. Basic fibroblast growth factor protects striatal neurons *in vitro* from NMDA-receptor mediated excitotoxicity. Brain Res 1992; 575: 351 – 5.

5 Mattson MP, Lovell MA, Furukawa K, Markesbery WR. Neurotrophic facors attenuate glutamate-induced accumulation of peroxides, elevation of intracellular calcium concentration and neurotoxicity and increase antioxidant enzyme activities in hippocampal neurons. J Neurochem 1995; 65: 1740 – 51.
6 Yamada K, Kinoshita A, Kohmura E, Sakaguchi T, Taguchi J, Kataoka K, *et al*. Basic fibroblast growth factor prevents thalamic degeneration after cortical infarction. J Cereb Blood Flow Metab 1991; 11: 472 – 8.
7 Koketsu N, Berlove DJ, Moskowitz MA, Kowall NW, Caday CG, Finklestein SP. Pretreatment with intraventricular basic fibroblast growth factor decrease infarct size following focal cerebral ischemia in rats. Ann Neurol 1994; 35: 451 – 7.
8 Nakata N, Kato H, Kogure K. Protective effects of basic fibroblast growth factor against hippocampal neuronal damage following cerebral ischemia in the gerbil. Brain Res 1993; 605: 354 – 6.
9 Regli L, Anderson RE, Meyer FB. Basic fibroblast growth factor increases cortical blood flow *in vivo*. Brain Res 1994; 665: 155 – 7.
10 Bethel A, Kirsch JR, Koehler RC, Finklestein SP, Traystman RJ. Intravenous basic fibroblast growth factor decrease brain injury resulting from focal ischemia in cats. Stroke 1997; 28: 609 – 16.
11 Fisher M, Meadows ME, Do T, Weise J, Trubetskoy V, Charette M, *et al*. Delayed treatment with intravenous basic fibroblast growth factor reduces infarct size following permanent focal cerebral ischemia in rats. J Cereb Blood Flow Metab 1995; 15: 953 – 9.
12 Ren JM, Kinklestein SP. Time window of infarct reduction by intravenous basic fibroblast growth factor in focal cerebral ischemia. Eur J Pharmacol 1997; 327: 11 – 6.
13 Xu W, Deng YF. Effect of anisodine on acute forebrain ischemia-reperfusion damage in rats. Acta Pharmacol Sin 1996; 17: 161 – 3.
14 Zhu XZ, Luo LG. Effect of nitroprusside (nitric oxide) on endogenous dopamine release from rat striatal slices. J Neurochem 1992; 59: 932 – 5.
15 Kuruvilla A, Cherian R, Theodore DR, Abraham J. Temporal profile of tissue levels of dopamine and its metabolites, HVA and DOPAC following focal cerebral ischemia in anaesthetized primates. Clin Exp Pharmacol Physiol 1986; 13: 519 – 24.

碱性成纤维细胞生长因子保护大鼠前脑抗缺血再灌注损伤

刘 璧, 朱兴族[1]
(中国科学院上海药物所药理一室, 上海 200031, 中国)

关键词 碱性成纤维细胞生长因子; 再灌注损伤; 视觉皮质; 多巴胺; 水; 钠; 钾; 高压液相色谱法; 纹状体

目的: 研究碱性成纤维细胞生长因子(bFGF)对大鼠脑缺血再灌注损伤的影响. **方法**: 电灼闭塞椎动脉并夹闭颈动脉, 使大鼠前脑缺血 20 分钟后, 放开双侧颈动脉再灌. 用高压液相色谱法测定纹状体中多巴胺(DA)类物质含量并用原子吸收分光光度法测定前脑中钠、钾含量. **结果**: 再灌 6 小时后, 纹状体中 DA 含量由对照的(99 ± 16) $\mu g \cdot g^{-1}$ (蛋白)减少至(70 ± 20) $\mu g \cdot g^{-1}$蛋白; 脑含水量由 77.34 % ± 0.19 %增加到 79.6 % ± 0.6 %; 钠含量由(9.3 ± 0.6) $mg \cdot g^{-1}$(脑干重)增加到(10.5 ± 0.6) $mg \cdot g^{-1}$(脑干重). 再灌开始后即刻静注 bFGF 45 $\mu g \cdot kg^{-1} \cdot h^{-1}$共 3 h 可阻止 DA 的减少和水、钠含量的升高. 组织学分析也表明 bFGF 可减轻神经元所受损伤. **结论**: bFGF 可以保护神经细胞对抗脑缺血再灌损伤.