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ACTIVATION OF ERK AND JNK MAP KINASES IN OPTIC NERVES OF RATS EXPOSED TO GLOBAL CEREBRAL ISCHEMIA

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SUMMARY

Objectives: *To determine the influence of global cerebral ischemia on the activation of extracellular-regulated kinases (ERK) and c-Jun N-terminal kinases (JNK) in optic nerves of rats exposed to different reperfusion periods.*

Materials and methods: *Transient global cerebral ischemia (20-min duration) was induced by the four-vessel occlusion method. After different reperfusion periods (5 and 10 min; 1; 6 and 12 h after ischemia), optic nerves were extracted and ERK and JNK activation signals were determined by Western immunoblot analyses.*

Results: *The activation signals of ERK and JNK were detected within first 10 min of reperfusion, but striking activation for both enzymes was found 1 h after ischemia. After a transient decrease, the activation of ERK returned to peak level after 12 h of reperfusion in the second wave of kinase activation. In that period, a slight increase of JNK activation was registered.*

Conclusion: *Our results demonstrated for the first time that ERK and JNK were activated in rat optic nerves during early and later periods of reperfusion, suggesting their potential active role in the response of cerebral white matter tissue to ischemic injury.*

Key words: *ERK – JNK - optic nerves - global cerbral ischemia - rat*

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INTRODUCTION

Extracellular-regulated kinases (ERK) and c-Jun N-terminal kinases or stress-activated protein kinases (JNK/SAPK) are members of the mitogen-activated protein (MAP) kinase family. They participate in intracellular signaling pathways that can initiate reparative processes or cell death (Irving et al. 2002, Lennmyr et al. 2002, Hwang et al. 2007). Activation of ERK is generally associated with cell survival, proliferation and differentiation (Shackelford et al. 2003) while activation of JNK is often correlated with cellular degeneration and apoptosis (Bonny et al. 2005, Guan et al. 2005). Recently, the effect of cerebral ischemia on MAP kinases has been reported in vitro and in vivo. According to these reports, ERK and JNK are activated during focal and global cerebral ischemia in neurons and microglia and are responsible for tissue injury after ischemia/ reperfusion (Aharon et al. 2004, Wang et al. 2004, Ho et al. 2007). The majority of studies have so far concentrated on the mechanisms of cerebral ischemic damage in gray matter since cerebral white matter was generally considered less vulnerable (Goldberg et al. 2003, Mrsic-Pelcic et al. 2004). Recent animal studies suggest that axons and oligodendroglia in white matter can be damaged even by brief ischemia (Schabitz et al. 2000). Despite recognized importance of the white matter damage caused by ischemia in different clinical conditions including some psychiatric disorders, little is known of the underlying mechanisms.

The objective of the present study was to determine the influence of 20-min global cerebral ischemia on activation of ERK and JNK in optic nerves of rats exposed to different reperfusion periods. To our knowledge, changes in activation of these kinases in optic nerves after global cerebral ischemia have not been reported.

MATERIALS AND METHODS

Animals

The study was carried out on Hannover-Wistar rats, weighing 200-250 g. Several experimental groups were included in the experiments, consisting of at least three animals randomly assigned to each group. With the exception of the control group (sham-operated animals), animals in all other experimental groups were exposed to global brain ischemia of 20-min duration. Following different reperfusion periods (5 or 10 min and 1; 6 or 12 h),the animals were sacrificed by decapitation and the optic nerves were rapidly excised and frozen at -80°C.

Procedure

Global cerebral ischemia was induced by the four-vessel occlusion method (Mrsic-Pelcic et al. 2004). Briefly, rat vertebral arteries were cauterized bilaterally and after 24 h of recovery, their common carotid arteries were occluded for 20 min. Rats that had lost their righting reflexes during the period of ischemia were assigned to the ischemic group. In the control group, the same surgical procedure was performed without interruption of blood flow.

Western blot analyses were performed as described in details elsewhere (Cerovac et al. 1999). Total protein (35 μg) (Bio Rad protein assay kit) was loaded for each sample onto a 12% polyacrylamide gel and run at 100 V. Transfer onto nitrocellulose (Bio-Rad, Canada) was conducted at 250 mA for 90 min. Membranes were incubated with primary polyclonal antibodies against ERK and JNK (Santa Cruz, CA) or antibodies against phosphorylated (active) MAPK (Promega, WI), and were used as recommended by the manufacturer. A horseradish peroxidase-conjugated secondary antibody (Amersham, UK) was utilized to allow detection of the appropriate bands using enhanced chemiluminescence reagent and film (Amersham, UK). All experiments were conducted a minimum of three times.

The intensities of representative bands were quantified by measuring the density of the immunoblots with Quantity One Bio Rad Image software for Windows.

RESULTS

The results of this study show moderate rise in ERK activation with two peaks occurring at 1h and 12 h of reperfusion (Figure 1B). The expression of ERK however remained constant throughout the periods of observation (Figure 1A).

In this study, activation of JNK was highest (three fold increase) 1 h after ischemia and was followed by a strong decrease thereafter, although 12 h after ischemia slight recovery of enzymatic activation was registered (Figure 2B). In contrast to ERK, the expression of JNK increased with time during the first hour of reperfusion with striking increase in signal expression after 12 h of reperfusion (Figure 2A).

DISCUSSION

Increased ERK activation has been reported in rodents after focal and global ischemia by several authors (Gu et al. 2001, Wang et al. 2004, Wakade et al. 2007). In most studies, maximal ERK activation was registered between 30 min and 2 h of reperfusion, althought signals could be detected even after 6 h or longer (Irving et al. 2002, Shackelford et al. 2003, Wang et al. 2004). The exact role of this activation in neuronal tissue is not clearly defined. Although several studies provided evidences for neuroprotective and antiapoptotic role of ERK in different cell lines (Gu et al. 2001, Lennmyr et al. 2002), recent observations showed that ERK activity is transiently increased in ischemic core and perifocal region before cell death in both focal and global ischemia (Wang et al. 2004). According to these

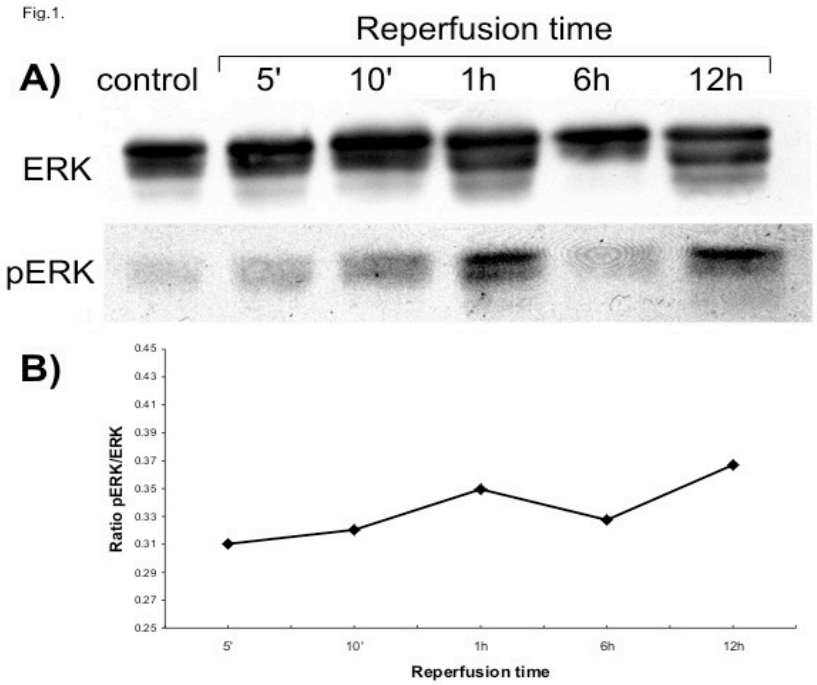

Figure 1. Expression and activation of ERK in optic nerves of animals exposed to different reperfusion periods

(A) Western immunoblots of total, non-phosphorylated (ERK) and active, phosphorylated (pERK) enzyme in optic nerve homogenates of control animals and ischemic animals exposed to different reperfusion intervals

(B) (B) Ratios between pERK and ERK were calculated as intensity of pERK divided by intensity of ERK and expressed as a fold increases in normalized phosphorylated ERK

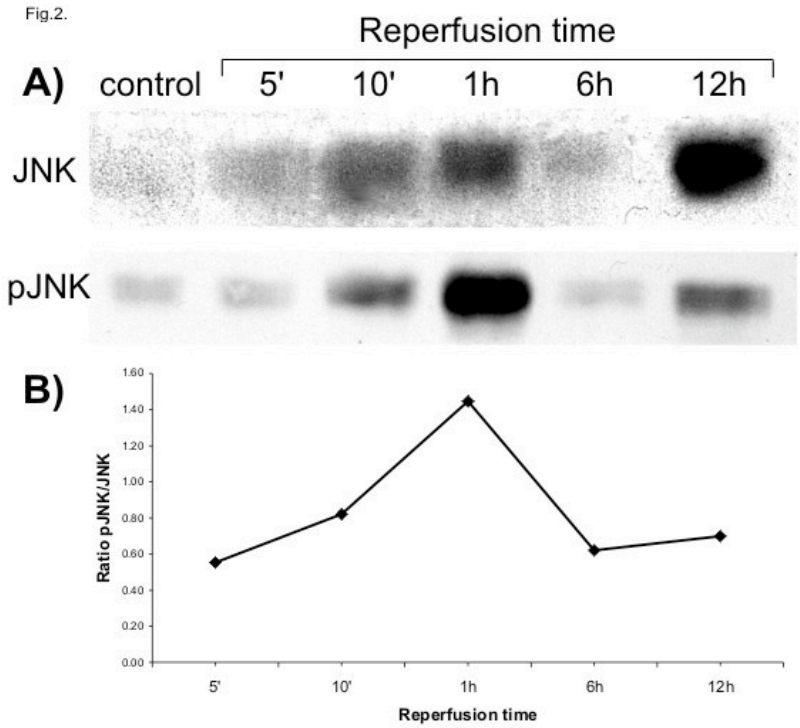

Figure 2. Expression and activation of JNK in optic nerves of animals exposed to different reperfusion periods
(A) Western immunoblots of total, non-phosphorylated (JNK) and active, phosphorylated (pJNK) enzyme in optic nerve homogenates of control animals and ischemic animals exposed to different reperfusion intervals
(B) Ratios between pJNK and JNK were calculated as intensity of pJNK divided by intensity of JNK and expressed as a fold increases in normalized phosphorylated JNK

reports elevated levels of phosphorylated ERK might be involved in the mechanisms underlying ischemia-induced cell death by mediating activation of proinflammatory cytokine IL-1ß (Wang et al. 2004) or by regulating the generation of reactive oxygen species (Yoo et al. 2005) in neurons and glia. Prolonged activation of ERK (up to 9 h) was found to contribute to glutamate induced oxidative stress in neuronal cell lines (Stanciu et al. 2000). It was pointed out that in tissues where ERK activation is detrimental to cell survival, cell death was brought about by oxidative stress. In our study the first peak of ERK activation was registered at 1 h of reperfusion, followed by slight decrease in activation. Second wave of increase in kinase activity was found 12 h after ischemia. Wheather such a dynamic in ERK activation represents tissue effort to reduce toxic effects of ischemia/reperfusion or contributes to tissue injury in early and later stages of reperfusion remains to be clarified in our further experiments.

Changes in total JNK protein could signify an increasing sensitivity of the JNK pathway to the stressful stimuli in early and later periods of reperfusion (Hayashi et al. 2000, Colangelo et al. 2004). Activation of JNK occurs in ischemia and it is often correlated with promotion of apoptosis and neuronal degeneration in different cell culture experiments (Takman et al. 2004) and in vivo models (Carboni et al. 2005, Li et al. 2005, Yatsusige et al. 2007). In models of focal and global cerebral ischemia, increased activity of JNK was detected in periods from 15 min to 24 h or even longer within tissues destined to die (Takagi et al. 2000, Jiang et al. 2007), suggesting that its activation may play a role in the mechanisms involved in ischemia-induced cell death. We assume that the striking increase in JNK activation registered in our study at 1h of reperfusion indicates a relatively early and strong tissue response to the ischemic insult. The slight rise in JNK activation 12 h after ischemia could be an indicator of JNK involvement in the tissue reaction to ischemic stimuli in later periods of reperfusion.

CONCLUSION

The present study demonstrated for the first time that ERK and JNK were activated in rat optic nerves during early and later periods of reperfusion, suggesting their potential active role in the response of cerebral white matter to ischemic injury. To clarify the exact role of this activation, further studies are required.

REFERENCES

1. *Aharon AS, Mulloy MR, Drinkwater DC, Lao OB, Johnson MD, Thunder M, Yu C, Chang P. Cerebral activation of mitogen-activated protein kinases after circulatory arrest and low flow cardiopulmonary bypass. Europ J Cardiothorac Surg. 2004; 26: 912-919.*
2. *Bonny C, Borsello T, Zine A. Targeting the JNK pathway as a therapeutic protective strategy for nervous system diseases. Rev Neurosci. 2005; 16: 57-67.*
3. *Carboni S, Antonsson B, Gaillard P, Gotteland JP, Gillon JY, Vitte PA. Control of death receptor and mitochondrial-dependent apoptosis by c-Jun N-terminal kinase in hippocampal CA1 neurones following global transient ischaemia. J Neurochem. 2005; 92: 1054-1060.*
4. *Cerovac Z, Ban J, Morinville A, Yaccato K, Shiver A, Maysinger D. Activation of MAPK by potassium bisperoxol(1,10-phenanthroline)oxovanadate (V). Neurochem Int. 1999; 34: 337-344.*
5. *Colangelo V, Gordon WC, Mukherjee PK, Trivedi P, Ottino P. Downregulation of COX-2 and JNK expression after induction of global ischemic tolerance in the gerbil brain. Brain Res. 2004; 1016: 195-200.*
6. *Goldberg MP, Ransom BR. New light on white matter. Stroke 2003; 34: 330-337.*
7. *Gu Z, Jiang Q, Zhang G. Extracellular signal-regulated kinase 1/2 activation in hippocampus after cerebral ischemia may not interfere with postischemic cell death. Brain Res. 2001; 901: 79-84.*
8. *Guan QH, Pei DS, Zhang QG, Hao ZB, Xu TL, Zhang GY. The neuroprotective action of SP600125, a new inhibitor of JNK, on transient brain ischemia/reperfusion-induced neuronal death in rat hippocampal CA1 via nuclear and non-nuclear pathways. Brain Res. 2005; 1035: 51-59.*
9. *Hayashi T, Sakai K, Sasaki C, Zhang WR, Warita H, Abe K. c-Jun N-terminal kinase (JNK) and JNK interacting protein response in rat brain after transient middle cerebral artery occlusion. Neurosci Lett. 2000; 284: 195-199.*
10. *Ho Y, Logue E, Callaway CW, DeFranco DB. Different mechanisms account for ERK activation in distinct brain regions following global ischemia and reperfusion. Neuroscience 2007; 145(1): 248-255.*
11. *Hwang MN, Kim KS, Choi YW, Jou I, Yoon S. PMA activates Stat3 in the Jak/Stat pathway and induces SOCS5 in rat brain astrocytes. Mol Cells 2007; 23(1): 94-99.*
12. *Irving EA, Bamford M. Role of Mitogen- and Stress-activated kinases in ischemic injury. J Cereb Blood Flow Metab. 2002; 22: 631-647.*
13. *Lennmyr F, Karlsson S, Gerwings P, Ata KA, Terent A. Activation of mitogen-activated protein kinases in experimental cerebral ischemia. Acta Neurol Scand. 2002; 106: 333-340.*
14. *Li CH, Wang RM, Zhang QG, Zhang GY. Activated mitogen-activated protein kinase kinase 7 redistributes to the cytosol and binds to Jun N-terminal kinase-interacting protein 1 involving oxidative stress during early reperfusion in rat hippocampal CA1 region. J Neurochem. 2005; 93: 290-298.*
15. *Mrsic-Pecic J, Pelcic G, Peternel S, Pilipovic K, Simonic A, Zupan G. Effects of the hyperbaric oxygen treatment on the Na,K-ATPase and superoxide dismutase activities in the optic nerves of global cerebral ischemia-exposed rats. Prog Neuro-Psychopharmacol Biol Psychiatry. 2004; 28: 667-676.*
16. *Schabitz WR, Fuhai L, Fischer M. The NMDA antagonist CNS 1102 protects cerebral gray and white matter from ischemic injury following temporary focal ischemia in rats. Stroke 2000; 31: 1709-1719.*
17. *Shackelford DA, Yeh RY. Activation of extracellular signal-regulated kinases (ERK) during reperfusion of ischemic spinal cord. Mol Brain Res. 2003; 115: 173-186.*
18. *Stanciu M, Wang Y, Kentor R, Burket N, Watkins S, Kress G, Reynolds I, Klann E, Angiolieri MR, Johnson JW, DeFranco DB. Persistent activation of ERK contrinutes to glutamate-induced oxidative toxicity in a neuronal cell line and primary cortical neuron cultures. J Biol Chem. 2000; 275: 12200-12206.*
19. *Takagi Y, Nozaki K, Sugino T, Hattori I, Hashimoto N. Phosphorylation of c-Jun NH2-terminal kinase and p38 mitogen-activated protein kinase after transient forebrain ischemia in mice. Neurosci Lett. 2000; 294: 117-120.*
20. *Takman R, Jiang H, Schaefer E, Levine RA, Lazarovici P. Nerve growth factor pretreatment attenuates oxygen and glucose deprivation-induced c-Jun amino-terminal kinase 1 and stress-activated kinases p38alpha and p38beta activation and confers neuroprotection in the pheochromocytoma PC12 model. J Mol Neurosci. 2004; 22: 237-250.*

21. *Wakade C, Khan MM, DeSevilla LM, Zhang QG, Mahesh VB, Brann DW. Tamoxifen neuroprotection in cerebral ischemia involves attenuation of kinase activation and SOD production and potentiation of mitochondrial SOD. Endocrinology 2007; Sep 27 (Epub ahead of print).*
22. *Wang ZQ, Wu DC, Huang FP, Yang GY. Inhibition of MEK/ERK 1/2 pathway reduces proinflammatory cytokine interleukin-1 expression in focal cerebral ischemia. Brain Res. 2004; 996: 55-66.*
23. *Yatsushige H, Ostrowski RP, Tsubokawa T, Colohan A, Zhang JH. Role of c-Jun N-terminal kinase in early brain injury after subarachnoid hemorrhage. J Neurosci Res. 2007; 85(7): 1436-1448.*
24. *Yiang HX, Guan QH, Pei DS, Zhang GY. Functional cooperation between KA2 and GluR6 subunits is involved in the ischemic brain injury. J Neurosci Res. 2007; 85(13): 2960-2970.*
25. *Yoo BK, Choi JW, Han BH, Kim WK, Kim HC, Ko KH. Role of MAPK/ERK 1/2 in the glucose deprivation-induced death in immunostimulated astroglia. Neurosci Lett. 2005; 376: 171-176.*

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