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BRAIN RESEARCH

Research Report

Action of citicoline on rat retinal expression of extracellular-signal-regulated kinase (ERK1/2)

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ABSTRACT

Citicoline is an essential endogenous intermediate in the biosynthesis of phosphatidylcholine, which acts as a therapeutic agent in models of central nervous system injury and neurodegenerative diseases. The present study investigated the effects of citicoline on extracellular-signal-regulated kinase 1/2 (ERK1/2) expression in the rat retina after kainic acid (KA) treatment. KA (6 nmol) was injected into the vitreous of the rat eyes. The animals were then injected intraperitoneally with citicoline (500 mg/kg) twice daily after the KA injection. The neuroprotective effects of citicoline were estimated by evaluating temporal changes in ERK1/2 using terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling (TUNEL), immunoblotting and immunohistochemical techniques. The expression of phosphorylated ERK1/2 was slightly decreased after 6 h, and significantly reduced after 12 h, in the rats receiving the KA injection plus citicoline treatment. Our results demonstrated that citicoline decreased the activation of ERK1/2 due to the KA treatment, suggesting that it exerts its neuroprotective activity by reducing the concentrations of proteins involved in apoptosis.

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1. Introduction

Citicoline is a naturally occurring endogenous nucleoside that is present in cells throughout the body. It is an essential intermediate in the synthesis of phosphatidylcholine, which is an important component of the neural cell membrane (Kennedy and Weiss, 1956; Trovarelli et al., 1981). Citicoline has been reported to produce neuroprotective effects in a variety of central nervous system models and neurodegenerative diseases (Secades and Frontera, 1995). In previous studies, citicoline has been shown to decrease infarct volume and edema and to improve cognitive performance after stroke, head trauma, Parkinson's disease and dementia (Spiers et al., 1996; Weiss, 1995). Recently, a few clinical studies demon-

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strated beneficial effects of citicoline on the function of the visual pathway in patients with retinal damage (Parisi et al., 1999). However, the mechanisms of the neuroprotection induced by citicoline after retinal damage have not been fully elucidated.

The signaling events that are implicated in survival, growth arrest or programmed cell death in response to DNA-damaging stress include the activation of mitogen-activated protein kinase (MAPK) pathways (Tang et al., 2002). MAPKs regulate a diverse array of functions, including neuronal survival, cell growth and proliferation (Boulton et al., 1991; Marshall, 1995; Segal and Greenberg, 1996), and apoptosis (Alessandrine et al., 1999; Bading and Greenberg, 1991). Members of the MAPK family include extracellular-signal-regulated kinase (ERK), c-jun NH_2-terminal protein kinase (JNK) and p38 MAPK. The latter two are strongly activated by cytokines, such as tumor necrosis factor-α (TNFα), as well as by a diverse array of stressors, including ultraviolet (UV) radiation and osmotic shock (Mielke and Herdegen, 2000). ERK is generally involved in cell proliferation and differentiation. The production of these survival signals could protect cells from ischemic damage. However, ERK is also activated after ischemia in the brain, heart, kidney and other tissues, and blocking ERK activation ameliorates ischemic injury (Irving and Bamford, 2002). These findings suggest that the activation of ERK might be a major signaling component of the retinal response to ischemia. The role of MAPK signaling pathways in regulating apoptosis during conditions of stress has been widely investigated. Recently, it was reported that the rate of apoptosis is regulated through the activation of MAPKs in retinal ischemia (Roth et al., 2003). However, although MAPK signaling cascades following the injection of kainic acid (KA) have been evaluated in the brain, relatively little is known about those in the retina. Thus, in the current study, we examined the effect of citicoline on MAPKs in the rat retina after the injection of KA.

2. Results

2.1. Expression and localization of ERK1/2 following KA injection

pERK immunoreactivity was upregulated after the KA injection, whereas no immunoreactivity was detected in the control retinas (Fig. 1A). Increased expression of pERK initially appeared in the inner plexiform layer (INL), inner plexiform layer (IPL) and ganglion cell layer (GCL) 6 h after the KA injection and was apparently localized in the Müller cell bodies (arrowheads) and the astrocytic processes (Fig. 1B, arrows). After 12 h, the immunoreactivity was largely seen in the Müller cell bodies, which vertically traversed the outer limiting membrane (OLM) to the inner limiting membrane (ILM) (Fig. 1C, arrows). Only faint immunoreactivity remained by 72 h (data not shown). Compared to the KA-treated retinas (Fig. 1C), treatment with citicoline after the KA injection markedly attenuated the pERK immunoreactivity in the INL, IPL and GCL after 12 h (Fig. 1D).

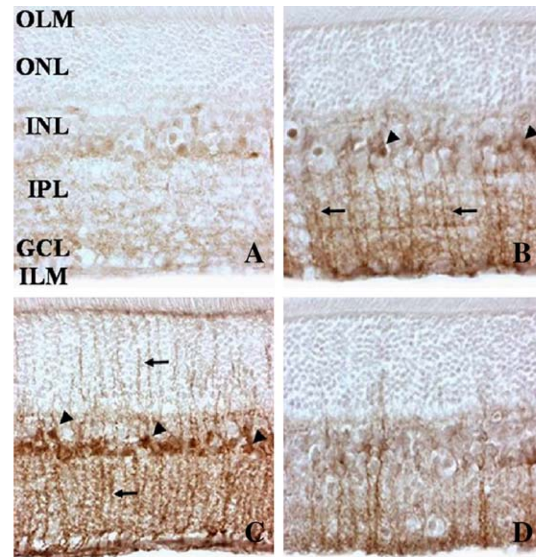

Fig. 1 – Representative photomicrographs showing the phosphorylated ERK immunoreactivity in a PBS-injected control (A), 6 h (B), and 12 h (C) after the KA injection and 12 h after the KA injection plus citicoline treatment (D). pERK1/2 immunoreactivity was evident in the Müller cells (arrowheads) and astrocytic projections (arrows) 6 h after the KA injection (B). At 12 h, pERK immunoreactivity was detected in processes extending from the ILM to the OLM (C), which substantially decreased 12 h after treatment with citicoline (D). GCL, ganglion cell layer; ILM, inner limiting membrane; INL, inner nuclear layer; IPL, inner plexiform layer; OLM, outer limiting membrane; ONL, outer nuclear layer. Original magnification, ×400.

2.2. Effect of citicoline on the change of the three MAPK family members (ERK, JNK and p38) after the KA injection

Western blotting analysis was carried out in order to confirm the change in the three MAPK family members (ERK, JNK and p38) using antibodies that recognize phosphorylated forms of these kinases in the rat retina after the KA injection. Two bands were detected in close proximity, with expected sizes corresponding to 44 (ERK1) and 42 (ERK2) kDa. The pERK level increased at 6 h (255 ± 16%) after the KA injection compared to the control rats, and the expression peaked at 12 h (283 ± 16%) and fell to below normal levels by 72 h after the KA injection (Fig. 2A). After 6 h, citicoline treatment slightly decreased the expression levels of pERK in the retina, whereas they were markedly decreased in the retinas of citicoline-treated rats after 12 h (Fig. 2A). By contrast, the expression levels of phosphorylated p38 and JNK after the KA injection were not decreased by treatment with citicoline (Figs. 2B, C). As an internal control, the α-tubulin level remained constant at all time points.

2.3. TUNEL study

We performed TUNEL staining in order to examine early cell death after the KA injection. In the normal rat retina, there

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