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BRAIN RESEARCH

Research Report

The increase in Zinc levels and upregulation of Zinc transporters are mediated by nitric oxide in the cerebral cortex after transient ischemia in the rat

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ABSTRACT

The transient occlusion of cerebral arteries causes an increase in zinc levels in the brain, which is associated with a production of nitric oxide (NO). The types of zinc transporters (ZnT) involved in zinc homeostasis in the cerebral cortex after hypoxia–ischemia are not completely known. We studied the effect of the transient occlusion (10 min) of the common carotid artery (CCA) on NO-induced zinc levels, ZnT mRNA expression, and cell-death markers in the cerebral cortex–hippocampus of the rat. Nitrites, zinc, and lipoperoxidation were quantified by colorimetric methods, ZnT expression was determined by RT-PCR, caspase-3 by ELISA and immunohistochemistry, and histopathological alterations by H&E staining. After restoration of the blood flow, the basal levels of NO and zinc increased in a biphasic manner over time, but the peaks of NO levels appeared earlier (2 h and 24 h) than those of zinc (6 h and 36 h). Upregulation of ZnT1, ZnT2, and ZnT4 mRNAs was determined after 8-h postreperfusion, but ZnT3 RNA levels were unaffected. Lipoperoxidation and caspase-3 levels were also increased, and necrosis and apoptosis were present at 24 h postreperfusion. All the effects determined were prevented by L-nitro-arginine methyl ester injected 1 h before the occlusion of the CCA. Our results suggest that the upregulation of ZnT1, ZnT2, and ZnT4 was to decrease the cytosolic zinc levels caused by NO after transient occlusion of the CCA, although this was unable to lead to physiological levels of zinc and to prevent cell damage in the cerebral cortex–hippocampus of the rat.

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1. Introduction

Zinc is one of the most abundant transition metals in the brain (Yeiser et al., 2002). It is a cofactor for a great variety of enzymes and plays a structural role in many proteins (Cuajungco and Lees, 1998; Ebadi and Iversen, 1994; Frederickson and Bush, 2001; Koh, 2001). The total zinc concentration in the brain was estimated to be approximately 150–200 μmol/L (10 μg/g wet brain tissue, estimated by autoradiogram) (Markesbery et al., 1984; Takeda, 2000), distributed extracellularly in fluids (500 nmol/L) (Weiss et al., 2000) and intracellularly in both cytosol (50–500 pmol/L) and synaptic vesicles (>1 mmol/L, depending on the cellular type) (Frederickson et al., 2000). Zinc found in synaptic vesicles in the hippocampus and cerebral cortex has been shown to be a neuromodulator of GABA and Glutamate postsynaptic effects (Danscher et al., 2001; Takeda, 2000; Westbrook and Mayer, 1987). Zinc homoeostasis is regulated by two large metal-transporter families; the Zip family that mediates zinc influx into the cytosol and the ZnT family that facilitates zinc efflux from the cytosol into intracellular cell compartments or out of the cell (Kambe et al., 2004). The ZnT family is formed of 10 proteins, also known as cation-diffusion facilitator (CDF) proteins (Cousins et al., 2006; Liuzzi and Cousins, 2004; Seve et al., 2004), whose presence of all ten members in the brain is not well-known (Feeney et al., 2005; Seve et al., 2004). ZnT1 and ZnT4 are located in the plasmatic membrane acting as zinc exporters to avoid an excessive rise of its intracellular concentration (Cousins and McMahon, 2000; McMahon and Cousins, 1998). ZnT2 is expressed at low levels in the brain and it has been associated with the vesicular zinc uptake (Seve et al., 2004). ZnT3 located in synaptic vesicles facilitates sequestration of zinc into synaptic vesicles (Palmiter et al., 1996b). ZnT5 is expressed abundantly in the brain and is thought to participate in the zinc uptake by the Golgi apparatus (Feeney et al., 2005; Seve et al., 2004; Suzuki et al., 2005). ZnT6 accumulates zinc in the Golgi apparatus (Huang et al., 2002; Kirschke and Huang, 2003) and it is moderately expressed in the brain (Feeney et al., 2005). To date, ZnT7, ZnT8, and ZnT9 have not been detected in the brain (Feeney et al., 2005) though they have been found in other tissues (Kambe et al., 2004; Seve et al., 2004). ZnT10 expression is restricted to fetal liver and brain and is thought to participate in the zinc efflux during fetal development (Seve et al., 2004).

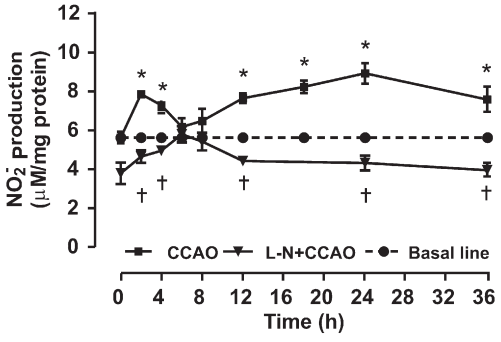

Fig. 1 – Increased nitric oxide production in the temporoparietal cerebral cortex–hippocampus after transient occlusion of the common carotid artery in the rat. Nitrite levels were determined by the Griess method. Each value represents the mean ± SEM of 5 independent experiments made in triplicate. CCAO = ischemia group with 10-min occlusion of the common carotid artery (CCA). L-N + CCAO = L-NAME administration 1 h before CCA occlusion. Dashed line shows basal values of the control group. * Significantly different from the control group; † significantly different from ischemia group. Student's t-test. Statistically different at $P \leq 0.05$.

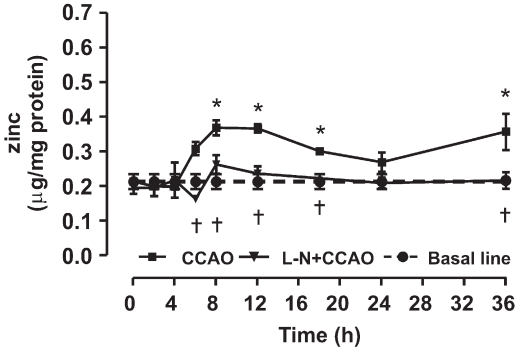

Fig. 2 – NO mediation of the zinc increase in the temporoparietal cerebral cortex–hippocampus after common carotid artery occlusion in the rat. Zinc levels were determined by the method of Johnson et al. (1977). Each value represents the mean ± SEM of 5 independent experiments made in triplicate. CCAO = ischemia group with 10-min occlusion of common carotid artery (CCA). L-N + CCAO = L-NAME administration 1 h before CCA occlusion. Dashed line shows basal values of the control group. * Significantly different from the control group; † significantly different from ischemia group. Student's t-test. Statistically different at $P \leq 0.05$.

Transient brain ischemia produces an increase in the intracellular zinc concentration, among other events, which can have an antioxidant effect or a cytotoxic role depending on its level (Bancila et al., 2004; Koh et al., 1996). The beneficial effect has been associated with the induction and regulation of metalloenzymes and transcription factors containing zinc-binding motifs such as zinc finger, zinc twist, zinc cluster, and RING finger (O'Halloran, 1993; Vallee et al., 1991). The harmful effect is possibly caused by accumulation of the free zinc in the cytoplasm because of the inability of ZnTs to efficiently remove it (Koh et al., 1996). This issue has been addressed only for the ZnT1 after transient forebrain ischemia (Tsuda et al., 1997), but the participation of the other ZnTs remains unknown. Another constant event of transient ischemia in the forebrain is the increase of nitric oxide (NO) in the cerebral cortex and hippocampus (Gonzalez-Barrios et al., 2002; Huang et al., 1994). The involvement of the three NO-synthase (NOS) isoforms was shown directly by enzymatic techniques and RT-PCR assays and pharmacologically by blockade assays using NOS inhibitors (Gonzalez-Barrios et al., 2002). All these strongly support a close association between NO and zinc in transient ischemia in the forebrain (Wei et al., 2004).

The linkage of zinc and NO has been directly demonstrated by studies in vitro and in vivo showing the ability of NO or NO-derived molecules to increase free zinc levels in cells (Bossy-Wetzel et al., 2004; Cuajungco and Lees, 1998) from oxidation