Exacerbation of Delayed Cell Injury After Transient Global Ischemia in Mutant Mice With CuZn Superoxide Dismutase Deficiency

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Background and Purpose—We have demonstrated that copper-zinc superoxide dismutase (CuZn-SOD), a cytosolic isoenzyme of SODs, has a protective role in the pathogenesis of superoxide radical–mediated brain injury. Using mice bearing a disruption of the CuZn-SOD gene (Sod1), the present study was designed to clarify the role of superoxide anion in the pathogenesis of selective vulnerability after transient global ischemia.

Methods—Sod1 knockout homozygous mutant mice (Sod1 −/−) with a complete absence of endogenous CuZn-SOD activity, heterozygous mutant mice (Sod1 +/−) with a 50% decrease in the activity, and littermate wild-type mice (male, 35 to 45 g) were subjected to global ischemia. Since the plasticity of the posterior communicating artery (PcomA) has been reported to influence the outcome of hippocampal injury, we assessed the relation between the plasticity of PcomAs and the decrease of regional cerebral blood flow in global ischemia.

Results—The fluorescence intensity of hydroethidine oxidation, a measurement of ethidium fluorescence for superoxide radicals, was increased in mutant mice 1 day after both 5 and 10 minutes of global ischemia, compared with wild-type mice. Hippocampal injury in the PcomA hypoplastic brains showed significant exacerbation in mutant mice compared with wild-type littermates 3 days after 5 minutes of global ischemia, although a marked difference was not observed at 1 day.

Conclusions—These data suggest that superoxide radicals play an important role in the pathogenesis of delayed injury in the vulnerable hippocampal CA1 subregion after transient global ischemia. (Stroke. 1999;30:1962-1968.)

Key Words: cerebral ischemia, global ■ free radicals ■ hippocampus ■ superoxide dismutase ■ superoxides ■ oxidative stress ■ mice

Oxygen radicals have been implicated in the pathogenesis of many neurological disorders and ischemic neuronal injury.1–3 One role of oxygen radicals appears to involve reperfusion after cerebral ischemia.4 Reperfusion supplies oxygen to the ischemic region, but oxygen could be a substrate for oxidative reaction producing oxygen radicals. Overproduced oxygen radicals that exceed the capacity of the endogenous antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase, and catalase, cause oxidative stress or injury in cerebral cells. Among these oxygen radicals, superoxide anions (O2−), being directly toxic to neurons,5,6 may initiate a free radical–mediated chain reaction causing additional central nervous system damage.3 We have demonstrated that copper-zinc SOD (CuZn-SOD), a cytosolic isoenzyme of SODs, has a protective role in the pathogenesis of superoxide radical–mediated brain injury, including cold injury–induced brain edema, traumatic brain injury, and focal transient ischemia/reperfusion.7–10

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The global ischemia model is well known as a model corresponding to clinical situations such as cardiac arrest and external circulation in cardiovascular surgery. The hippocampus, particularly the CA1 subregion, is known to be one of the brain areas vulnerable to transient cerebral global ischemia. The postischemic interval required for the apparent selective neuronal loss is inversely proportional to the severity or duration of the ischemic insult, the so-called maturation phenomenon described by Ito et al.11 This interval is especially prolonged in the hippocampal neurons,12 a process called delayed neuronal death,13 although its detailed mechanism is still unclear. An apoptotic pathway was reported to be involved in the mechanism of hippocampal injury after transient global ischemia.14,15 The mechanism underlying apoptosis is multifactorial and includes reactive oxygen
species as a major mediator.\textsuperscript{16–18} Our recent studies showed the increased production of $\text{O}_2^-$ after global ischemia in the vulnerable hippocampal CA1 subregion.\textsuperscript{19} The reduction of hippocampal injury after transient global ischemia in transgenic mice and rats further implicates the determinate role of overexpressed human CuZn-SOD in global ischemic injury.\textsuperscript{20,21}

The development of mice deficient in the mouse CuZn-SOD gene ($Sod1$) has provided a model for assessing the role of endogenous CuZn-SOD in nervous system injury.\textsuperscript{22} These mutant mice with a decrease in or a complete absence of endogenous CuZn-SOD activity were used in a dose-dependent study of CuZn-SOD activity in hippocampal injury after transient global ischemia. The present study was designed to clarify the protective role CuZn-SOD plays in the pathogenesis of selective vulnerability and delayed neuronal death after transient global ischemia.

**Materials and Methods**

*Sod1*-Deficient Mice

Mutant mice designated as 129/CD1 –$Sod1^{-/-}$ Cep\textsuperscript{1} were produced by Reaume et al\textsuperscript{22} at Cephalon, Inc (West Chester, Pa). These mutant mice were used to create mice that were either heterozygous ($Sod1^{+/+}$) or homozygous ($Sod1^{-/-}$) mutants. These mutant mice with the 129/CD1 background were bred with the CD1 strain of mice for at least 8 generations. There were no distinct phenotypes between these mutants and the wild-type animals. CuZn-SOD activity in the mutant mice was assessed by the method of nondenaturing gel electrophoresis assay, as previously described.\textsuperscript{23} These mutant mice and age-matched wild-type littermates generated from heterozygous parents ($Sod1^{+/-}$) were subjected to global ischemia as detailed below. All animals were treated in accordance with Stanford University guidelines and the animal protocol approved by Stanford University’s Administrative Panel on Laboratory Animal Care.

Global Cerebral Ischemia

Global ischemia was induced by bilateral common carotid artery (CCA) occlusion under controlled ventilation. Mice were anestheitized with chloral hydrate (350 mg/kg IP) and xylazine (4 mg/kg IP). The endotracheal tube was inserted, and respiration was controlled with the use of an animal ventilator with inspiratory stroke volume of 0.5 mL and a respiratory rate of 120 breaths per minute. Rectal temperature was maintained at 37.0±0.5°C with a homeothermic blanket. The bilateral CCA was exposed, and temporary clips were applied. After a period of ischemia, the clips were removed, and atropine sulfate (0.5 mg/kg) was injected intraperitoneally to reverse respiratory and blood pressure that were depressed by the chloral hydrate. The animals were cared for in individual cages at 20°C.

Measuring Regional Cerebral Blood Flow

Change in regional cerebral blood flow (rCBF) was evaluated in both groups of animals with a laser Doppler flowmeter (LASERFLO BPM\textsuperscript{2}, Vasomedic). A probe was placed on the skull above the middle cerebral artery territory cortex (0.5 mm posterior and 4 mm lateral from bregma). The rCBF was monitored continuously from 5 minutes before until 5 minutes after induction of ischemia. Decreased rates of rCBF during bilateral CCA occlusion were calculated as (ischemia rCBF/preischemia rCBF)×100.

Carbon Black Evaluation of Plasticity of the Posterior Communicating Arteries

Since the plasticity of the posterior communicating arteries (PcomAs) would influence the outcome of hippocampal injury after transient global ischemia, we assessed the plasticity of the PcomAs before the histological evaluation of the hippocampal injury. The animals were anesthetized with ketamine and xylazine. After perfusion fixation through the ascending aorta with 10 U/mL heparin in 0.9% saline and 3.7% formaldehyde in PBS, carbon black ink in an equal volume of 20% gelatin in H$_2$O was injected. The removed brains were fixed in 3.7% formaldehyde overnight at 4°C. Plasticity of PcomAs was graded by a scale of 0 to 3. Since the contralateral PcomA would make a collateral flow in the ipsilateral hippocampus, the plasticity scores of the PcomAs in both hemispheres were totaled, then the brains with scores of 0 to 3 were classified as the hypoplastic PcomA group and the brains with sums $>3$ were classified as the normal PcomA group.

**In Situ Detection of Superoxide Production**

The spatial production of superoxide anion ($O_2^-$) in cerebral ischemia was investigated by the method of in situ detection of oxidized hydroethidium (Molecular Probes).\textsuperscript{24–26} Hydroethidium solution (200 μL; 1 mg/1 mL PBS with 1% dimethylsulfoxide) was administered intravenously 1 hour before the animals were killed. Hydroethidium rapidly penetrated into the brain, which was selectively oxidized to ethidium by $O_2^-$. Animals were killed 1 day after global ischemia by transcardiac perfusion as described above. After post-fixation in 3.7% formaldehyde, brains were sectioned to a 50-μm thickness at the level of the hippocampus with the use of a vibratome and placed on glass slides (Superfrost, Fisher Scientific). Fluorescence of ethidium was observed with a microscope (Axioplan2, Zeiss) at excitation of 510 to 550 nm and emission $>580$ nm. To analyze the fluorescence signal of hydroethidium, photomicrographs ($×650$) were scanned by a GS-700 imaging densitometer (Bio-Rad), and then the signal intensity was measured in 7 to 9 individual cells in each group with the use of Multi-Analyst software (Bio-Rad).\textsuperscript{26}

**Histological Analysis of Hippocampal Injury**

Brain samples of the PcomA hypoplastic group were chosen for histological analysis of hippocampal injury on the basis that complete ischemia was induced without a supply of collateral flow from posterior circulation. The brains were sectioned to a 50-μm thickness with a vibratome and stained with cresyl violet. Neuronal damage in the hippocampus was qualitatively evaluated on the basis of a scoring system of 0 to 4, as described by Murakami et al\textsuperscript{21}: grade 0, no damage to any hippocampal subregion; grade 1, scattered ischemic neurons in CA1 subregion; grade 2, moderate ischemic damage in CA1 subregion; grade 3, whole pyramidal cells damaged in CA1 subregion; and grade 4, extensive cell damage in all hippocampal subregions. Neuronal damage was evaluated by a researcher blinded to the studies.

**Results**

CuZn-SOD Activity in Mutant Mice

Nondenatured gel electrophoresis followed by nitroblue tetrazolium staining confirms that CuZn-SOD activity is reduced in $Sod1^{-/-}$ and completely absent in $Sod1^{+/-}$ mutants (Figure 1). These results were well matched with our previous study in which we reported that the CuZn-SOD activity of blood and brain tissue in this strain was reduced to 50% in $Sod1^{-/-}$ mutants and to only a trace in $Sod1^{+/-}$ mutants.\textsuperscript{27}

**Physiological Conditions and Mortality After Ischemia**

The results of measuring rCBF showed a significant difference in the decreased rate of rCBF during global ischemia between the PcomA hypoplastic group (mean±SD,
between these groups. *P < 0.01, ANOVA; Figure 2). However, within the hypoplastic group, there was no significant difference in rCBF during ischemia between wild types and Sod1 knockout mutants. No significant difference was seen in PcomA plasticity between the hypoplastic group of either genotype (Table 1). The mortality of each group is shown in Table 2. No significant difference was observed in the mortality data. Three days after 5 minutes of global ischemia, the mortality was higher in the Sod1 +/- mutants than in the wild types and was highest in the Sod1 +/- mutants. We did not perform 10 minutes of global ischemia insult in the Sod1 homozygous knockout mutant mice because we expected the mortality to be too high for these animals compared with the 50% mortality with 5 minutes of global ischemia.

**Production of O$_2^-$ in the Hippocampal CA1 Subregion**

The expression of oxidized hydroethidium signals as a red color, which was consistent with our previous report, shows production of O$_2^-$ in the hippocampal CA1 pyramidal cells after global ischemia. Under normal physiological conditions, oxidized hydroethidium signals were detected as small particles in the cytosol, indicating the leakage of O$_2^-$ produced in the mitochondria. One day after 5 minutes of global ischemia, scattered cells in the Sod1 +/- mutants showed cytosolic oxidized hydroethidium signals (Figure 3B); however, no marked changes were observed in the wild types (Figure 3A). After more severe ischemia, such as 10 minutes of global ischemia, the red particles of oxidized hydroethidium signals were increased slightly, but not the cytosolic expression, in the wild types (Figure 3C). On the other hand, almost all pyramidal cells markedly showed cytosolic oxidized hydroethidium signals in the CA1 subregion of Sod1 knockout mice (Figure 3D). Mean intensity of hydroethidium signal was significantly higher in mutant mice 1 day after 5 minutes of ischemia (1.384 ± 1.539; mean optical density ± SD) than in wild-type mice (0.194 ± 0.143) (P < 0.05). After 10 minutes of ischemia, hydroethidium signals in the CA1 subregion were significantly increased in both groups, and mutant mice showed significantly higher signals (4.619 ± 0.704) than wild-type mice (1.902 ± 1.393) (P < 0.001). The results that showed increased superoxide radical signals in mutant mice in the postischemic CA1 subregion are considered to have occurred because of the 50% reduction of Sod1 activity in the mutant mice compared with the wild-type mice.

**Hippocampal Injury After Mild and Severe Global Ischemia**

Various levels of severity of neuronal damage in the hippocampus were seen after global ischemia, but the ischemic

<table>
<thead>
<tr>
<th>Ischemia</th>
<th>Genotype</th>
<th>1 Day</th>
<th>3 Days</th>
</tr>
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<tbody>
<tr>
<td>5 minutes</td>
<td>Wild type</td>
<td>0% (0/9)</td>
<td>12.5% (1/8)</td>
</tr>
<tr>
<td>Sod1 +/-</td>
<td>0% (0/6)</td>
<td>25.0% (2/8)</td>
<td></td>
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<tr>
<td>Sod1 +/-</td>
<td>0% (0/5)</td>
<td>50.0% (3/6)</td>
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</tr>
<tr>
<td>10 minutes</td>
<td>Wild type</td>
<td>0% (0/8)</td>
<td>27.3% (3/11)</td>
</tr>
<tr>
<td>Sod1 +/-</td>
<td>14.3% (1/7)</td>
<td>25.0% (2/8)</td>
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Each value represents percent mortality (number of dead animals/number of operated animals) of each genotype and time course.
damage was hardly observed in the brains of the group with normal plasticity of the PcomA. To equalize the anatomic background between Sod1 knockout mutants and wild types, the brains of the PcomA hypoplastic group were used for histological assessment of hippocampal injury after global ischemia. Representative photographs showed morphological features of injured brains and the hippocampus by cresyl violet staining 3 days after 5 minutes of global ischemia in wild types and Sod1 knockout mutants. Although no remarkable change was observed in the wild types, ischemic changes were observed in scattered neurons of the CA1 subregion in the Sod1 +/- mutants. Furthermore, severe damage and edema were observed in the hippocampal CA1 subregion, dentate gyrus, and cortex in Sod1 +/- mice (Figure 4). A qualitative analysis shows neuronal damage of the hippocampus in wild types and Sod1 knockout mutants 1 and 3 days after 5 and 10 minutes of global ischemia (Figure 5). No significant difference was observed 1 day after 5 minutes of ischemia between each genotype. Hippocampal damage was exacerbated in Sod1 +/- mutants and was significantly more severe in Sod1 +/- mutants compared with wild types 3 days after 5 minutes of ischemia. After 10 minutes of global ischemia, hippocampal damage was greater in Sod1 +/- mutants than in wild types at 1 day; however, there was no significant difference between mutants and wild types 3 days after ischemia.

**Discussion**

Targeted disruption of the Sod1 gene results in the partial or complete loss of CuZn-SOD activity in mutant mice. This study shows that the level of production of O$_2^-$ increased after global ischemia in the vulnerable hippocampal CA1 subregion, especially in the mutant mice with a reduction in CuZn-SOD activity. In addition, the present study demonstrated that the hippocampal injury was exacerbated in mutant mice after transient global ischemia. Furthermore, this protective effect of CuZn-SOD was obtained in both acute injury after relatively intense ischemia and in progressive injury after mild ischemia. These findings are consistent with the hypothesis that an increased level of O$_2^-$ mediates these pathologies after transient global ischemia.

Gerbils have been widely used for global ischemia studies on the basis that bilateral CCA occlusion can induce almost complete forebrain ischemia without the reduction of collat-
general blood flow from posterior circulation because of the lack of PcomAs as a connection between anterior and posterior circulation. Several models of global ischemia in mice have been proposed, such as 3-vessel occlusion and unilateral occlusion with systemic hypoxia. We previously reported on a simple mouse model of transient global cerebral ischemia with systemic hypoxia.21,28 In this report, we used the PcomA hypoplastic group to evaluate the ischemic change if the contralateral PcomA was plastic as well. Blood flow would come through azygous anterior cerebral arteries. Therefore, we totaled each score of the bilateral PcomAs and classified them as PcomA hypoplastic brains and normoplastic brains with the cutoff point at 3. We then used the PcomA hypoplastic group to evaluate the hippocampal damage after ischemia. The validity of this classification was confirmed by measuring rCBF during ischemia. Using this method, we observed a hippocampal injury after transient global ischemia in mutant mice with a CuZn-SOD deficiency. The mean scores of PcomA plasticity and the decreased levels of rCBF did not show a significant difference between wild types and Sod1 knockout mutants (Table 1), indicating that the ischemic condition induced by the present method was at the same level in each group of animals.

Our previous study showed the production of O2− in the hippocampal CA1 subregion after global ischemia in rats with the use of in situ imaging of superoxide by hydroethidium oxidation.20 It was then suspected that production of O2− increased in mutant mice with CuZn-SOD deficiency. In the present study, we clearly show that the O2− signals increased in Sod1 mutants compared with wild types. As previously observed,25,29 O2− signals were shown by oxidized hydroethidium as small particles in the cytosol, suggesting mitochondrial production of O2−. Under normal conditions, these signals were observed without any significant difference in either group (data not shown). After mild ischemia, diffuse cytosolic expression of O2− signals was observed in scattered cells in the hippocampal pyramidal cells in Sod1 +/− mice (Figure 2B); therefore, after severe ischemia, a marked, diffuse cytosolic expression of O2− signals was seen in pyramidal cells in the CA1 subregion of Sod1 +/− mutants (Figure 3D). On the other hand, a particle-like expression of O2− signals increased, but a diffuse expression was not observed in wild types (Figure 3C). These findings indicate that O2−, which was overproduced in the mitochondria and leaked into the cytosol, was trapped by CuZn-SOD in wild types. However, in Sod1 mutant mice, it exceeded the capacity of CuZn-SOD, and then the O2− signals filled the cytosol of pyramidal cells in the vulnerable hippocampal CA1 subregion after global ischemia.

Mutant mice with a CuZn-SOD deficiency exhibited a significant increase in damage in a dose-dependent manner in the hippocampus after transient global cerebral ischemia. Hippocampal damage tended to be exacerbated in Sod1 mutants but was not significant 1 day after relatively mild ischemia (5 minutes). However, the mortality (Table 2) and the hippocampal damage (Figures 4 and 5) were markedly exacerbated in reverse proportion to CuZn-SOD activity 3 days after mild ischemia. With these data, we propose that CuZn-SOD plays a protective role in delayed hippocampal damage after mild global ischemia, which conforms to the results of global ischemia in CuZn-SOD transgenic rats.20 However, after 10 minutes of global ischemia, which is a relatively severe ischemia, the hippocampal damage was significantly exacerbated in Sod1 +/− mutants 1 day after ischemia, although severe damage was observed in wild types 3 days after ischemia. These findings suggest that CuZn-SOD has a protective effect against acute damage after severe ischemic insult, and these findings are supported by the results we previously reported showing that neuronal damage was decreased in Sod1 transgenic mice after focal ischemia8,10 and was exacerbated in Sod1 knockout mutants.27 Since Sod1 was reported to delay neuronal apoptosis in vitro,30 we do not completely rule out the possibility that the earlier development of the hippocampal damage in Sod1 knockout mutants might be due to an interference with CuZn-SOD activity. However, CuZn-SOD deficiency was significantly exacerbated in Sod1 +/− mutants 1 day after ischemia, although severe damage was observed in wild types 3 days after ischemia. These findings suggest that CuZn-SOD has a protective effect against acute damage after severe ischemic insult, and these findings are supported by the results we previously reported showing that neuronal damage was decreased in Sod1 transgenic mice after focal ischemia8,10 and was exacerbated in Sod1 knockout mutants.27 Since Sod1 was reported to delay neuronal apoptosis in vitro,30 we do not completely rule out the possibility that the earlier development of the hippocampal damage in Sod1 knockout mutants might be due to an interference with CuZn-SOD activity.
knockouts may contribute, in part, to the marked exacerbation of the hippocampal injury after global ischemia in the present study (Figure 5). A more detailed time course study would address this important issue.

In the present study, the mechanisms of O$_2^-$ that mediate hippocampal injury after transient global ischemia are still unclear. Recent studies, although still somewhat controversial, have identified some apoptotic features by biochemical and morphological evidence such as terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) staining, internucleosomal DNA fragmentation, as indicated by the DNA laddering pattern. Several apoptosis-regulatory genes and enzymes are found to be induced in ischemic cells. Caspase-3, which is in the interleukin 1β converting enzyme family of cytokine proteins, was reported to have an important effect in apoptotic cell death. The caspase-3 mRNA and activated form of the protein were predominantly increased in hippocampal CA1 neurons after transient global ischemia. Furthermore, ventricular infusion of z-DEVD-fmk, a caspase-3 inhibitor, decreased cell death and DNA fragmentation in the CA1 subregion after global ischemia.

We have previously reported that Sod1 knockouts showed a marked increase of the amount of DNA fragmentation and of infarction volume after transient focal cerebral ischemia in mice and that DNA damage was reduced in the hippocampal CA1 subregion in Sod1 transgenic rats after global ischemia, together suggesting that reactive oxygen species could exacerbate DNA damage and infarction after ischemia/reperfusion. In the present study, we have shown the first evidence of the dose-dependent effect of endogenous SOD on the increase of hydroethidium signals and on the hippocampal injury after global cerebral ischemia. As for DNA damage after global ischemia, our recent study showed that the downregulation of the DNA repair enzyme apurinic/apyrimidinic endonuclease (APE/Ref-1), which plays a central role in the base excision repair pathway by providing a 3`-OH primer for repair synthesis of DNA after all types of oxidative damage, is implicated in apoptotic cell death after global ischemia. Although the cell death pathway, through either necrosis or apoptosis, is still unclear after transient global ischemia, DNA fragmentation was observed after global ischemia insults. These data again suggest that oxidative DNA damage may cause hippocampal damage after global ischemia. Future studies, such as DNA damage indicated by TUNEL staining, DNA laddering, caspase-3 activation, and downregulation of APE/Ref-1 expression in Sod1 knockout mice after transient global ischemia, are considered necessary to address this critical issue.

In conclusion, selective knockout of Sod1 gene reduced CuZn-SOD activity and decreased neuronal damage exacerbated by CuZn-SOD activity in the hippocampal CA1 subregion after transient global ischemia. Our results support the hypothesis that CuZn-SOD plays an important role in neuronal damage in vulnerable regions after transient global ischemia. Overexpression of CuZn-SOD by gene transfer may have therapeutic implications in preventing delayed neuronal damage in patients who sustain stroke or cardiac arrest.

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References
The accompanying article from Kawase and collaborators uses mutant mice deficient in the cytosolic or CuZn form of SOD to demonstrate the important dose-dependent role of this enzyme in protecting the hippocampal CA1 subregion from the delayed injury caused by exposure to a transient (5- to 10-minute) global ischemia produced by occlusion of the CCA. While there is already much evidence supporting the importance of superoxide in the injury caused by exposure of hippocampal neurons to transient ischemia and reperfusion, the results of the present study demonstrate the important dose-dependent role of this enzyme in protection against hippocampal damage.

There appear to be at least several key processes through which superoxide anion can participate in tissue injury. First, superoxide anion is a key source of production of more reactive oxidants that directly cause damage to key organelles and constituents of tissues. Highly reactive oxidant species can result from processes including the generation of peroxynitrite by the reaction of superoxide with nitric oxide and from the reductive release of bound iron by superoxide and the subsequent generation of hydroxyl radical–like oxidants from reactions of ferrous iron with peroxides. In addition, superoxide anion appears to cause mitochondrial dysfunction associated with the disruption of iron-sulfur centers in the electron transport chain. This and other actions of superoxide-derived oxidants appear to be key processes that result in apoptosis. Thus, superoxide anion has multiple ways of promoting tissue injury processes.

CuZn-SOD is one of the 3 major types of SOD that are present in mammalian tissues. This form of SOD is primarily located in the cytosol of cells, and its principal function appears to be the removal of superoxide from this intracellular compartment. The Mn form of SOD is essentially a mitochondrial enzyme, and it is thought to metabolize superoxide generated within the mitochondria in a manner that efficiently prevents the detection of superoxide release from this organelle. The third form of SOD is an extracellular enzyme (EC-SOD), which is thought to scavenge superoxide in the extracellular environment of cells that secrete this enzyme. While there is evidence that superoxide can be transported across membranes, the extent to which the scavenging of superoxide made in one compartment by SOD that is present in another compartment provides protection against aspects of cellular injury is an area that is very poorly understood. There is substantial evidence that each form of these enzymes is located. This suggests that each form of SOD participates in the protection of tissues from the pathophysiological effects of oxidant stress in the regions where these enzymes are located. This suggests that each form of SOD is likely to have protective roles against aspects of tissue injury processes activated by ischemia and reperfusion. The results of the present study demonstrate the important protective role of the scavenging of superoxide by the CuZn form of SOD, which is located primarily in the cytosol of hippocampal cells.

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