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MITOCHONDRIAL SUPEROXIDE PRODUCTION AND MnSOD ACTIVITY FOLLOWING EXPOSURE TO AN AGONIST AND ANTAGONISTS OF IONOTROPIC GLUTAMATE RECEPTORS IN RAT BRAIN

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Abstract - The involvement of NMDA and AMPA/kainate receptors in the induction of superoxide production in the rat brain was examined after intrahippocampal injection of kainate, a non-NMDA receptor agonist; kainate plus CNQX, a selective AMPA/kainate receptor antagonist; or kainate plus APV, a selective NMDA receptor antagonist. The measurements took place at different times in the ipsi- and contralateral hippocampus, forebrain cortex, striatum, and cerebellum homogenates. The used glutamate antagonists both ensured sufficient neuroprotection in the sense of lowering superoxide production and raising MnSOD levels, but in the mechanisms and time dynamics of their effects were different. Our findings suggest that NMDA and AMPA/kainate receptors are differentially involved in superoxide production.

Key words: APV, CNQX, excitotoxicity, kainate, neuroprotection, oxidative stress

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INTRODUCTION

The physiological effects of glutamate, the most important excitatory neurotransmitter in the brain, are mediated by ionotropic and metabotropic receptors. Glutamate receptors are believed to exist as oligomers of ionotropic receptor subunits or as dimers of metabotropic receptors. Both receptor types can be divided into several families based on sequence identity and pharmacological, electrophysiological, and biochemical characteristics (Skaper *et al.* 2001). Ionotropic glutamate receptors comprise N-methyl-D-asparate (NMDA), alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA), and kainate (KA) receptors (Varju *et al.* 2001). Mammals show six NMDA receptor subunits, four AMPA receptor subunits, and five KA receptor subunits (Janssens *et al.* 2001).

Oxidative stress and excessive activation of glutamate receptors are converging processes and represent sequential as well as interacting factors that provide a final common pathway for cell vulnerability in the brain (Coyle *et al.* 1993). While excitotoxic and oxidative injury may occur independently, growing evidence indicates that reactive oxygen species formation may also be a specific consequence of glutamate receptor-mediated neurotoxicity (Dugan *et al.* 1995). The precise cellular mechanisms that lead to neurotoxicity under these conditions still remain unclear. Although NMDA receptors likely contribute critically to neuronal injury in various acute conditions, several observations support the hypothesis that AMPA/KA receptors may be of greater importance to the neurodegenerative process (Carriedo *et al.* 1998, 2000). Considerable evidence supports a link between Ca^{2+} influx and glutamate receptor-mediated neurodegeneration. Brief periods of activation of highly Ca^{2+}-permeable NMDA channels can result in substantial intracellular Ca^{2+} accumulation and widespread neuronal injury (Lu *et al.* 1996, Hyrc *et al.* 1997, Tseng *et al.* 2003). Mitochondria can buffer these large Ca^{2+} loads, but they do so at the expense of triggering injurious reactive oxygen species (ROS) production (Peng *et al.* 1998). Additionally, the extremely rapid interconversion of ROS within the cell can make it difficult to identify the originating species. In contrast to NMDA receptors, AMPA/KA receptors are generally

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Ca^{2+}-impermeable and trigger injury more slowly, with prolonged periods of activation needed before significant neuronal injury occurs (Koh *et al.* 1990). Subpopulations of central neurons, however, are highly vulnerable to AMPA/KA receptor-mediated injury, likely attributable in part to the expression of large numbers of AMPA/KA channels with high Ca^{2+} permeability (Weiss *et al.* 1994).

Glutamate neurotoxicity is mediated by reactive oxygen species formed as a consequence of several processes, including nitric oxide (Alabadi *et al.* 1999, Nakaki *et al.* 2000, Radenović *et al.* 2003, Halasz *et al.* 2004) and superoxide (Li *et al.* 2001) production. Superoxide radicals react rapidly with nitric oxide to form highly cytotoxic peroxynitrite, which acts through lipid peroxidation (Lee *et al.* 2001). Although there are a number of intracellular sources of free radicals, the mitochondria are thought to be the most important (Ciriolo *et al.* 2001). The bioenergetic properties of *in situ* mitochondria play a central role in controlling the susceptibility of neurons to acute or chronic neurodegenerative stress. Mitochondria from different tissue sources display differential susceptibility to oxidizing species (Cassarino *et al.* 1999; Heales *et al.* 1999). However, it is now becoming apparent that, within the brain, there is a differential susceptibility of various brain cell types to oxidizing species. In contrast to astrocytes, neurons appear to be particularly vulnerable to the action of free radicals. Such vulnerability may arise from an inability to sustain cellular energy demands by glycolysis and an inferior capacity to handle oxidizing species (Sengpiel *et al.* 1998). Regional distribution of NMDA and AMPA/KA receptors of the rat brain was found to be highest in deep layers (layer 5) of the forebrain cortex, cerebellar granule cell layer, and caudate putamen (Bailey *et al.* 2001), which is why we tested these particular brain regions: hippocampus, forebrain cortex, striatum, and cerebellum.

The Mn-containing superoxide dismutase (SOD) isoenzyme is predominantly localized to neurons and their processes throughout the brain and the spinal cord (Lindenau *et al.* 2000). It seems reasonable to conclude that differences in the basal content of SOD-isoenzymes may contribute to different cellular susceptibilities in neurodegenerative processes that are accompanied by oxidative stress. Mitochondrial MnSOD seems to be a key enzyme in oxygen metabolism in the brain, and it is considered to be a major factor in protection of nervous tissue against excitotoxic and ischemic/hypoxic lesion (Budd *et al.* 1996, 1997). Manganese-containing SOD represents a ROS-inducible enzyme which should allow the adaptation of brain cells to variation in ROS concentrations resulting from their oxidative metabolism (Gonzales-Zulueta *et al.* 1999).

In order to investigate the role of NMDA and AMPA/KA receptors in glutamate neurotoxicity, we studied neuroprotective effects of respective receptor antagonists by monitoring the production of superoxide anion and activity of mitochondrial MnSOD in the hippocampus, forebrain, cortex, striatum, and cerebellum after intrahippocampal injection [KA, a non-NMDA receptor agonist; KA plus 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a selective AMPA/KA receptor antagonist; or KA plus 2-amino-5-phosphonopentanoic acid (APV), a selective NMDA receptor antagonist].

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MATERIALS AND METHODS

Animals

Adult rats of the Wistar strain (*Rattus norvegicus*) of both sexes, with body weight of 200 ± 30 g, were used for experiments. Groups of two or three rats per cage (Erath, FRG) were housed in an air-conditioned room at room temperature of 23 ± 2°C with 55 ± 10% humidity and with lights on 12 h/day (07:00-19:00). The animals were given a commercial rat food and tap water ad libitum. These animals were anesthetized by giving intraperitoneal injections of sodium pentobarbital (0.0405 g/kg b.w.) and were placed in a stereotaxic frame.

Experimental procedure and intracerebral injection of drugs

The rats were divided into five basic groups (three with drug treatment: KA, KA+CNQX, KA+APV; and two control: intact animals and sham-operated), each basic group consisting of five different subgroups (according to survival times) and each subgroup consisting of eight animals. Animals in the drug-treated groups received an unilateral injection of only KA (Sigma Chemical Co. U.S.A., 0.5 mg/ml, dissolved in 0.1 M saline, pH 7.2; 1 μL total volume); KA plus CNQX (Wak-Chemie Medical GMBH, Tocris, 0.5 mg/ml, dissolved in DMSO, pH 7.2; 1 μL total volume); or KA plus APV (Sigma Chemical Co. U.S.A., 0.5 mg/ml, dissolved

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in 0.1 M saline, pH 7.2; 1 µL total volume) into the CA3 region of the hippocampus (coordinates from bregma: anteroposterior: - 3.3 mm, dorsoventral: 3.2 mm, and lateral: 3.0 mm) using a Hamilton microsyringe with a beveled tip. The control group received the same volume (1 µL), but only saline solution (sham-operated), while the group of intact animals served as a control for mechanical injection. The animals were allowed to survive for 5 min to seven days (5 min, 15 min, 2 h, 48 h, and 7 days). All animals were anesthetized and decapitated, after which the brains were immediately removed. The ipsi- and contralateral hippocampus, forebrain cortex, striatum, and cerebellum from individual animals were quickly isolated and homogenized in ice-cold buffer containing 0.25 M sucrose, 0.1 mM EDTA, and 50 mM K-Na phosphate buffer, pH 7.2. Homogenates were centrifuged twice at 1580g for 15 min at 4^0C. The supernatant (crude mitochondrial fraction) obtained by this procedure was then frozen and stored at - 70^0C.

Superoxide production and measurement

In these experiments, superoxide was measured by monitoring the reduction of nitro blue tetrazolium (NBT) as previously described (Spitz *et al.* 1989). Along with other tetrazolium salts, NBT salts are chromogenic probes useful for superoxide determination. Detection of this product was by spectrophotometric quantification of a colored formazan product formed from blue tetrazolium. Reduction of NBT was measured at 5[illegible] nm.

Superoxide dismutase assay

The assay of MnSOD activity by the adrenaline method (Fridovich *et al.* 19[illegible]) was used. The method is based on measurement of the degree of adrenaline autooxidation inhibition by MnSOD contained in the examined samples (50 µL) in 50 mM sodium carbonate buffer, pH 10.2, with 5 mM KCN. Enzymatic activity was expressed in units per milligram of protein.

Protein concentration measurement

The content of protein in the rat brain homogenates (hippocampus, striatum, forebrain cortex, and cerebellum, ipsilateral and contralateral) was measured by the method of Lowry *et al.* (1951) using bovine serum albumin (Sigma) as standard. All measurements were performed in triplicate.

MATERIALS

Chemicals were purchased from Sigma (St. Louis, Mo., U.S.A.). Other chemicals were of analytical grade. All drug solutions were prepared on the day of the experiment.

Animals used for procedures were treated in strict accordance with the NIH Guide for Care and Use of Laboratory Animals (1985).

Data presentation and analysis

All experiments were done with n = 8. Each assay was performed at least twice under identical conditions. Data are expressed as means ± SD. The statistical significance of differences between groups was assessed by Student's *t*-test (paired and unpaired) for individual comparisons and regression analysis for overall significance (with $p < 0.05$ as significant and $p < 0.01$ as very significant).

RESULTS

Superoxide production and MnSOD activity in rat brain

The results presented in Figs. 1-4 show the superoxide levels (O_2^-, µM NBT/mg protein) and the MnSOD activity (MnSODx10^3 IU/mg protein) in ipsilateral hippocampal, cortical, striatal, and cerebellar homogenates, respectively. Results obtained comparing superoxide and MnSOD levels in the intact group of animals and sham-operated animals show the effect of mechanical injection in rat brain. There was no significant difference between superoxide and MnSOD levels in these two groups. This means that mechanical injection alone is not sufficient to trigger oxidative stress and/or excitotoxicity. We therefore considered sham-operated animals as controls. In the control group, superoxide production and MnSOD levels showed no significant differences between the left and right hemispheres in any of the tested structures. Also, there was no significant difference between mean superoxide levels and MnSOD levels obtained from each hemisphere after antagonist treatment in any of the tested brain structures, even when the injection site was in the ipsilateral hippocampus (results not presented). The used antagonists of glutamate receptor showed the same pattern in all tested brain structures.

Intrahippocampal injection of KA resulted in genera-

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lly higher levels ($p<0.05$) of superoxide production in all tested brain structures. The obtained levels of superoxide production were highest in the hippocampus. Rapid increase in superoxide production was found at 5 min after KA injection, and these higher levels continued to be above normal at all tested times (with 7 days as the final time point) in all tested brain structures (Fig. 1). At 15 min after KA injection, superoxide measurements in the hippocampus (11.72 ± 1.15 μM NBT/mg protein), in the forebrain cortex (10.46 ± 1.19 μM NBT/mg protein), and in the striatum (10.36 ± 1.18 μM NBT/mg protein) showed statistically very significant differences ($p<0.01$) compared with the equivalent control group (Figs. 1-3). The results obtained for the contralateral hippocampus, forebrain cortex, and striatum were similar (data not shown).

Levels of MnSOD were highest in the hippocampus, at the injection site (control or KA-treated). Intrahippocampal injection of KA caused significant increase in MnSOD levels after 5 and 15 min, followed by further significant increase at 48 h and 7 days in the hippocampus and in the forebrain cortex (Figs. 1 and 2), and an approximately similar effect was found in the cerebellum (Fig. 4). In the striatum significant increase in MnSOD levels was found only at an early tested time frame, at 5 min (Figs. 3 and 4).

Intrahippocampal injection of KA plus CNQX resulted in very significant decrease ($p<0.01$) of superoxide production, below the control levels, in all tested brain structures compared with the equivalent group of KA-treated animals (Figs. 1-4). The effect of this antagonist is striking at 5 min from injection in all tested brain structures (2.53 ± 1.13 μM NBT/mg protein in the hippocampus, 2.76 ± 1.10 μM NBT/mg protein in the forebrain cortex, 2.59 ± 1.19 μM NBT/mg protein in the striatum, and 2.25 ± 1.08 μM NBT/mg protein in the cerebellum, $p<0.01$; Figs. 1-4). Measurement at 5 min after KA plus CNQX injection showed significant decrease ($p<0.05$) of superoxide levels compared to the control group in all tested brain structures.

Intrahippocampal injection of KA plus CNQX resulted in significant increase of MnSOD activity only at late testing times (2 h, 48 h, and 7 days) in nearly all tested brain structures compared with the equivalent group of KA-treated animals and the control group (Figs. 1-4). The effect of this antagonist was striking 7 days from injection in all tested brain structures, but MnSOD activity was highest in the hippocampus (4.23 ± 0.26 MnSODx10^3 IU/mg protein, $p<0.01$; Fig. 1).

Intrahippocampal injection of APV plus KA resulted in a reduction of superoxide levels back to control levels in all tested brain structures (Figs. 1-4). Thus, there was significant decrease in superoxide levels only in comparison to KA-treated animals ($p<0.05$). Analogous to the excitotoxic effect obtained with KA-injected animals, statistically the most significant decrease was obtained at 15 min (5.37 ± 1.19 μM NBT/mg protein in the hippocampus, 5.76 ± 1.11 μM NBT/mg protein in the forebrain cortex, $p<0.01$; Figs. 1-4).

Intrahippocampal injection of KA plus APV resulted in a biphasic effect on MnSOD activity in all tested structures (Figs. 1-4). This antagonist caused significant increase in MnSOD levels after 5 and 15 min, followed by further significant increase at 48 h and 7 days in all tested brain structures in comparison to the control. Compared to KA-treated animals, the most significant increase in MnSOD activity was at 7 days after injection in all tested brain structures (Figs. 1-4), with the highest levels of MnSOD activity measured in the forebrain cortex (4.06 ± 0.19 MnSODx10^3 IU/mg protein, $p<0.01$; Fig. 4). The used antagonist of glutamate receptor showed the same pattern in all tested brain structures.

Behavioral changes after glutamate antagonist injection

The purpose of this study was to investigate fine changes in superoxide and MnSOD levels during the process of excitotoxicity in various brain parts. Our aim was to inject glutamate antagonists (appropriate dose), but to avoid any behavioral changes ("wet dog shake", focal seizure of the limbs and neck, hypersalivation, or generalized convulsion) and typical limbic seizures evolving into status epilepticus. We were careful to avoid it because during status epilepticus hippocampal blood flow, oxygen supply, and body temperatures are modified. These effects are accompanied by severe damage to all subfields of the hippocampal formation. It is a condition of intense metabolic activation and could interfere with our results and measurements. We did not measure epileptic activity by electroencephalogram. All animals in the experiment behaved normally.

DISCUSSION

Recent studies have linked glutamate toxicity and

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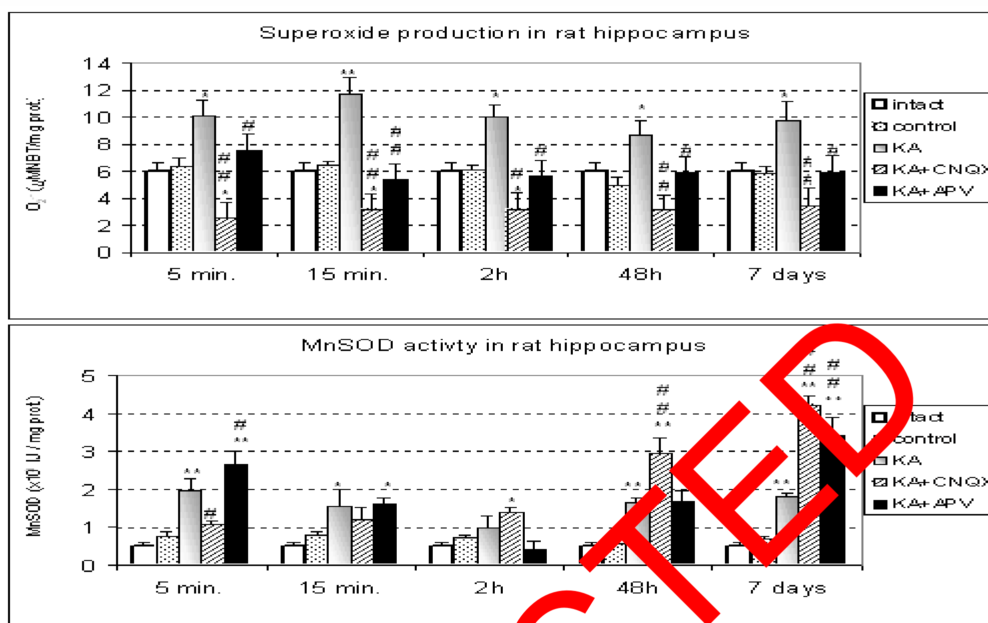

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Fig. 1. Effect of intrahippocampal injection of kainate (KA), kainate plus APV (KA+APV), and kainate plus CNQX (KA+CNQX) on superoxide levels (O_2', μM NBT/mg proteins) and MnSOD activity ($x10^3$ IU/mg prot.) in the rat ipsilateral hippocampus at different survival times. Data are means ± S.D. * and ** indicate statistically significant (p<0.05) and very significant (p<0.01) differences between glutamate antagonists treated and control (sham-operated) animals. # and ## indicate statistically significant (p<0.05) and very significant (p<0.01) differences between glutamate antagonist-treated and kainate-treated animals.

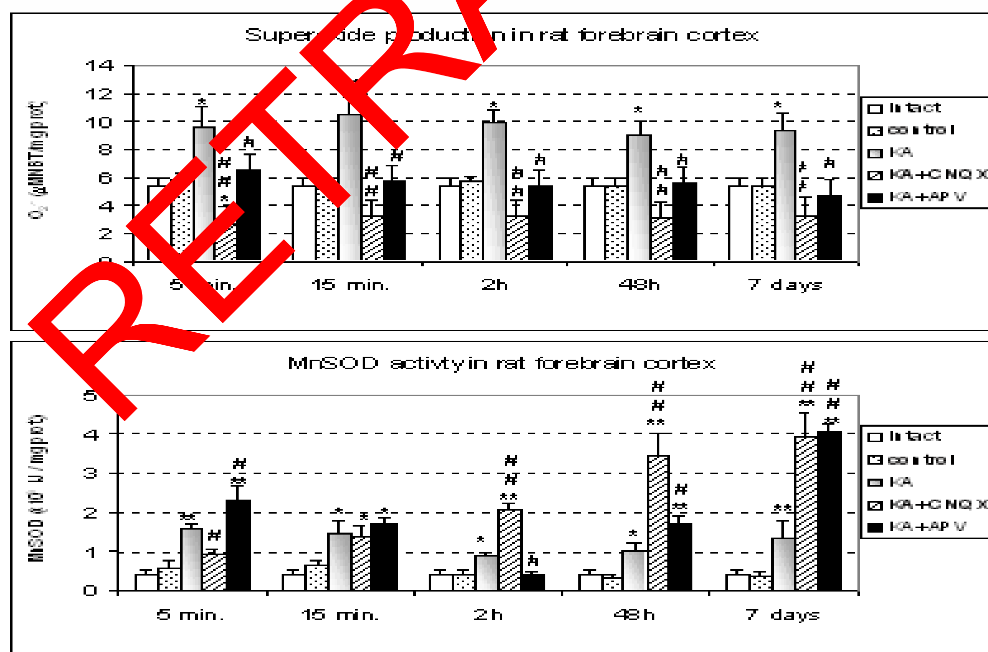

Fig. 2. Effect of intrahippocampal injection of kainate (KA), kainate plus APV (KA+APV), and kainate plus CNQX (KA+CNQX) on superoxide levels (O_2', μM NBT/mg proteins) and MnSOD activity ($x10^3$ IU/mg prot.) in the rat ipsilateral forebrain cortex at different survival times. Data are means ± S.D. * and ** indicate statistically significant (p<0.05) and very significant (p<0.01) differences between glutamate antagonist-treated and control (sham-operated) animals. # and ## indicate statistically significant (p<0.05) and very significant (p<0.01) differences between glutamate antagonist-treated and kainate-treated animals.

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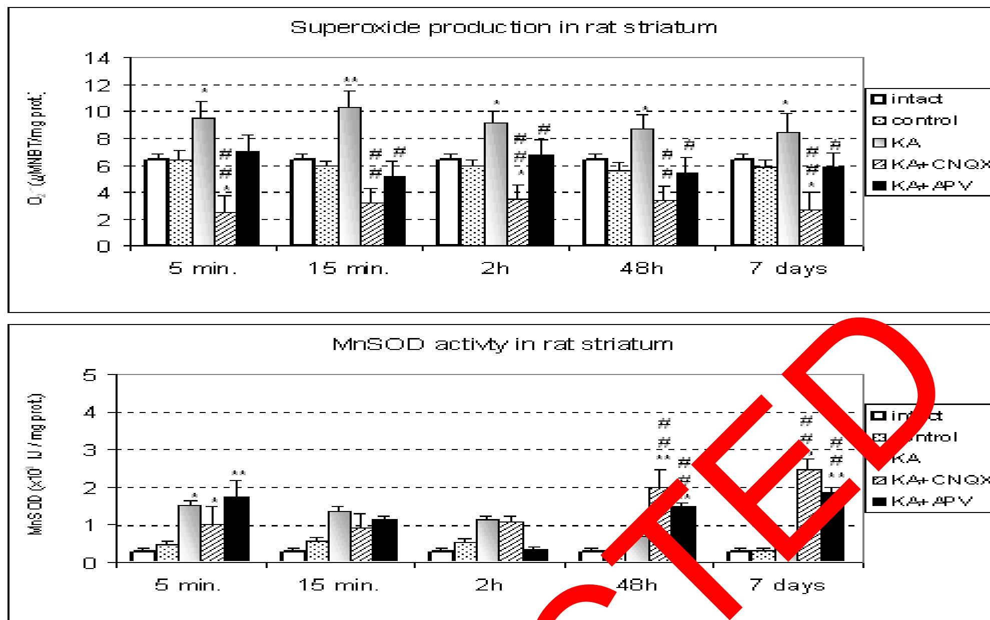

Fig. 3. Effect of intrahippocampal injection of kainate (KA), kainate plus APV (KA+APV), and kainate plus CNQX (KA+CNQX) on superoxide levels (O$_2$', μM NBT/mg proteins) and MnSOD activity (x10^3 IU/mg prot.) in the rat ipsilateral striatum at different survival times. Data are means ± S.D. * and ** indicate statistically significant (p<0.05) and very significant (p<0.01) differences between glutamate antagonist-treated and control (sham-operated) animals. # and ## indicate statistically significant (p<0.05) and very significant (p<0.01) difference between glutamate antagonist-treated and kainate treated animals.

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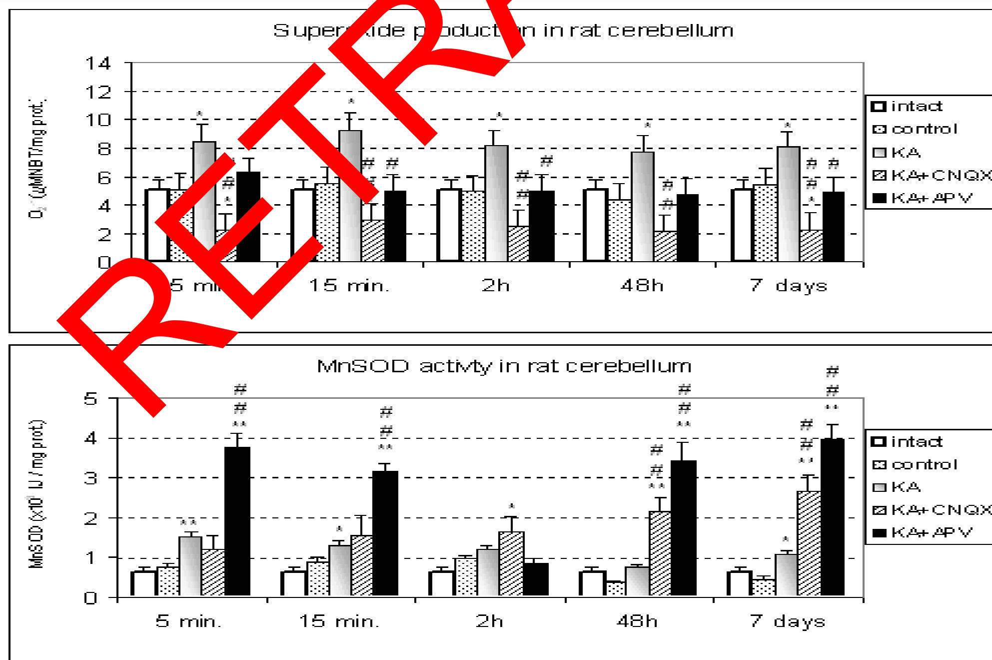

Fig. 4. Effect of intrahippocampal injection of kainate (KA), kainate plus APV (KA+APV) and kainate plus CNQX (KA+CNQX) on superoxide levels (O$_2$', μM NBT/mg proteins) and MnSOD activity (x10^3 IU/mg prot.) in the rat ipsilateral cerebellum at different survival times. Data are means ± S.D. * and ** indicate statistically significant (p<0.05) and very significant (p<0.01) differences between glutamate antagonist-treated and control (sham-operated) animals. # and ## indicate statistically significant (p<0.05) and very significant (p<0.01) differences between glutamate antagonist-treated and kainate treated animals.

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superoxide production to mitochondrial disfunction (Dugan *et al.* 1995). A number of synthetic glutamate receptor antagonists, especially drugs interfering with NMDA receptors, were identified as promising neuroprotective agents, but they failed in clinical trials because of undesirable side effects or lack of efficacy (Urenjak *et al.* 2000). There is a pharmacological difference between glutamate and its receptor agonists. Unlike its receptor agonists, glutamate can activate all glutamate receptors and can be taken up by many different cells, including neurons and glia. The glutamate transport system may play a role in the difference between glutamate and its receptor agonists in the observed excitotoxic effects (Li *et al.* 1998). These glutamate transporters can be inhibited by peroxynitrite, formed by a combination of superoxide and nitric oxide and sensitive to the cell's redox state (Trotti *et al.* 1997). Reduced glutamate uptake will prolong glutamate receptor activation by extracellular glutamate, which will exacerbate neuronal injury (Trotti *et al.* 1996). Under physiological conditions, a dynamic equilibrium exists in vivo between the oxidative damage potential and the antioxidant defense capacity. However, during episodes of oxidative stress, increased free radical production or reduced antioxidant reservoirs might upset this balance. Superoxide radical is much less reactive than other ROS and can cross cell membranes and act at a distance. As the first protective mechanism, superoxide dismutase (SOD) reacts with superoxide to produce hydrogen peroxide and molecular oxygen.

In the present study, an appropriate dose of KA (0.5 mg/ml) was used to cause minor brain damage in the ipsilateral, but not contralateral hippocampus with no behavioral or epileptic effects. We have shown that superoxide levels in the rat brain increased immediately after kainate injection and remained increased throughout the experiment (7 days was the longest survival time examined). This significant increase in superoxide production correlated with MnSOD levels and presumably with the degree of neuronal injury. Neuronal cells responded to oxidative stress in kainate-induced neurotoxicity and induced the protective mechanism to increase MnSOD levels. The striatum, the main component of the basal ganglia, receives glutamatergic inputs from the cortex and thalamus, and considerable attention has therefore been given to the role of excitotoxicity in striatal disorders. A KA-induced effect was demonstrated in all tested brain structures, while the striatum was shown to be the most resistant to KA-induced injury according to our results.

Induction of mitochondrial MnSOD under pathological conditions is variable and related mainly to the type of injury (Bidmon *et al.* 1997; Kim *et al.* 2000; Liang *et al.* 2000). Neuronal superoxide production varies with metabolic activity and age. The role of oxygen radicals in AMPA/KA receptor-mediated injury is less clear. Developmental increase in mitochondrial superoxide production and oxidative DNA damage following KA seizures suggests that mitochondrial oxidative stress may be a key factor that renders the developing brain resistant to seizure-induced brain damage (Patel *et al.* 2003).

Agonist-triggered Ca^{2+} influx may constitute a key link between glutamate receptor activation and subsequent neurodegeneration. In cortical culture, brief periods of activation of NMDA channels, which are highly Ca^{2+}-permeable, are capable of triggering widespread neurodegeneration. In contrast, much more prolonged periods of activation of AMPA/KA receptor-gated channels are required before comparable neurotoxicity develops. This may reflect the fact that most AMPA/KA channels are poorly permeable to Ca^{2+} and likely cause secondary Ca^{2+} influx via the depolarization and activation of voltage-sensitive Ca^{2+} channels. Multiple factors have been hypothesized to contribute to the differences in toxicity that result from NMDA and AMPA/KA receptor activation (Carriedo *et al.* 1996; Nicholls *et al*, 2000).

In the present study, we detected a differential effect of the NMDA antagonist APV and the AMPA/KA antagonist CNQX on superoxide production and MnSOD activity after intrahippocampal injection with KA. The effect of KA on superoxide production was completely blocked by the glutamate antagonists. Intrahippocampal injection of KA with APV resulted in decrease of superoxide production to around control levels in all tested brain structures. Thus, significant decrease in superoxide levels was found only in comparison to KA-treated animals, *i.e.* the overall effect of a selective NMDA receptor antagonist was a decrease of kainate-induced excitotoxicity. The accent effect of intrahippocampal injection of KA plus the selective AMPA/KA receptor antagonist CNQX resulted in significant decrease of superoxide production to below the control levels in all tested brain structures, indicating the existence of an AMPA/KA receptor-mediated component of basal superoxide production in control conditions. The effect of this antago-

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nist is striking at 5 min from injection in all tested brain structures.

It has been shown that MnSOD has a neuroprotective role in glutamate excitotoxicity (Keller *et al.* 1998). and is characterized by a heterogeneous distribution in the brain (Akai *et al.* 1990). In the striatum, cholinergic neurons and somatostatin neurons are enriched with MnSOD, as are cholinergic neurons of the basal forebrain (the latter highly so). In the hippocampus, MnSOD enrichment is mainly observed in parvalbumin-containing neurons. The presence of MnSOD-positive interneurons was recorded in the stratum piramidale with highest packing densities in the subiculum and CA3. The highest packing density within the hippocampal formation occurred in the polymorphic cell layer of the dentate gyrus. It is also possible that MnSOD is involved in limiting the damage in remote brain areas that were not ischemic caused by scavenging radicals formed in response to deafferentiation (Bidmon *et al.* 1997).

Our results show that there is a clear, transient increase of inducible MnSOD in all tested brain regions after intrahippocampal injection of glutamate antagonists. Although NMDA-induced superoxide production was blocked selectively with APV, still a rapid increase in MnSOD levels (within 5 min) was found in the ipsilateral and the contralateral areas, which possibly receive a few direct connections from the lesioned area, after KA plus APV injection. It is clear from the data that at the onset MnSOD upregulation was higher in the hippocampus, suggesting that the hippocampus may be intrinsically more protected from toxic effects by MnSOD than other areas. The first increase in MnSOD activity occurred between 5 min and 2 h. The obtained increase is due to activation of MnSOD by increased levels of superoxide (Gonzales-Zulueta *et al.* 1999; Li *et al.* 1998). The delayed increases of MnSOD activity at 48 h and 7 days suggest rapid *de novo* synthesis probably involving transcription of the gene and translation of its mRNA (Hussain *et al.* 2004).

From the data presented it is obvious that increase in MnSOD activity in KA-induced excitotoxicity is not dependent on superoxide production only. We hypothesize that by selectively blocking AMPA receptors with CNQX, we reduced superoxide production but did not inhibit mitochondrial transport or several other cellular pathways for radical generation. A possible explanation is that MnSOD activity can be induced by many cytokines and not only by superoxide itself (Pinteaux *et al.* 1996). The effect of increased MnSOD activity was obtained with KA+CNQX intrahippocampal injection, but the CNQX and APV effects differed in time dynamics. In all tested brain structures, significant increase was detected from 2 h up to 7 days. Previous studies suggested that mitochondrial MnSOD is important for resistance to toxic cellular insults and plays a major protective role (Nicholls *et al.* 1999). It seems that the mechanisms and the time points of induction of MnSOD activity by NMDA and AMPA/KA antagonists may be different. Most probably direct injury leads to an instant induction of MnSOD expression, whereas more time is needed to transfer the signal via afferents or efferents to the remote regions. The used glutamate antagonists APV and CNQX both ensured sufficient neuroprotection in the sense of lowering superoxide production and raising MnSOD levels, but the mechanisms and time dynamics of their effects were different.

Here we record that induction of MnSOD occurs in disparate brain areas (hippocampus, forebrain cortex, striatum, and cerebellum) after intrahippocampal glutamate antagonist-induced seizure. This finding indicates that protection against superoxide radicals took place not only around the lesioned area, but may spread to more remote areas. Furthermore, the data point to a differential role of NMDA and AMPA/KA receptors during this neuropathological condition. The increase of MnSOD in distinct brain regions that are functionally connected via afferents and efferents suggests that these regions are affected by the injury. It suggests that MnSOD protects the cells in these regions from superoxide-induced damage and therefore may limit the retrograde and anterograde spread of neurotoxicity.

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СТВАРАЊЕ СУПЕРОКСИДА И АКТИВНОСТ МИТОХОНДРИЈАЛНЕ MnSOD ПОСЛЕ ПРИМЕНЕ АГОНИСТА И АНТАГОНИСТА ЈОНОТРОПНИХ ГЛУТАМАТНИХ РЕЦЕПТОРА У МОЗГУ ПАЦОВА

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Интрацеребрална апликација каиничне киселине, агонисте глутаматних рецептора, у селективно осетљив CA3 регион хипокампуса пацова доводи до ексцитотоксичног оштећења неурона у овој структури, посредованог стварањем слободних радикала као медијатора оштећења. Узимајући у обзир анатомско-функционалну повезаност селективно осетљивих можданих структура (хипокампус, кортекс, стријатум и церебелум) и специфичност њихове биохемијске организације, мерењем концентрације супероксидног радикала и MnSOD праћено је просторно и временско ширење ексцитотоксичног оштећења индукованог интрахипокампалном апликацијом каиничне киселине. Полазећи од овако изазване ексцитотоксичности испитивана је могућност неуропротективног деловања APV-селективног антагонист NMDA рецептора и CNQX-потентног антагонисте AMPA/каинатних рецептора, односно супстанци које на различитим местима прекидају каскаду оштећујућих реакција. Доказана је различита улога јонотропних NMDA и AMPA/каинатних глутаматних рецептора у продукцији супероксида и модулације и активности митохондријалне MnSOD. Показано је да оксидативни стрес који мења хомеостазу нервних ћелија може бити ублажен применом различитих компетитивних антагониста јонотропних глутаматних рецептора (APV и CNQX), али да је њихов механизам дејства различит, што имплицира неуропротективно дејство ових супстанци.

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