Changes of Neuronal Transmission in the Hippocampus After Transient Ischemia in Spontaneously Hypertensive Rats and the Protective Effects of MK-801

Kazuhiko Suyama, MD

Background and Purpose: I studied the mechanism of postischemic neuronal degeneration in the hippocampus by an electrophysiological method.

Methods: Sequential changes of field potentials evoked by perforant path stimulation in the dentate gyrus and the CA1 region of the hippocampus were evaluated in spontaneously hypertensive rats up to 7 days after transient global ischemia induced by bilateral occlusion of the carotid arteries for 20 minutes after electrocauterization of the vertebral arteries. Animals were treated with vehicle or the excitotoxin antagonist (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-amine (MK-801, 2 mg/kg or 5 mg/kg) intraperitoneally 30 minutes before ischemia.

Results: Complete recovery of the population spike was observed in the dentate gyrus within 24 hours after recirculation, followed by a gradual reduction of population spike amplitude. In contrast, population spike in the CA1 region showed partial recovery 24 hours after recirculation, and an abrupt reduction of population spike amplitude occurred on day 2. There was no significant enhancement of population spike amplitude in either region throughout the experiment. Interneuronal recurrent inhibition in the dentate gyrus was enhanced on day 4, and ischemic changes were apparent in the CA1 pyramidal cells on day 7. Pretreatment with 5 mg/kg MK-801 prevented field potential and pathological changes completely in the dentate gyrus and partially in the CA1 region.

Conclusions: My results indicate that pathological changes of the CA1 pyramidal neurons after transient ischemia may not be the result of postischemic overstimulation. However, neuronal transmission in the CA1 region may be persistently impaired during or after transient ischemia. (Stroke 1992;23:260-266)

The mechanisms of neuronal damage after transient global ischemia or hypoxia have been extensively studied.1-3 Excessive release of excitatory amino acids from presynaptic neurons to extracellular space during ischemia4-7 may activate the postsynaptic N-methyl-D-aspartate (NMDA) subtype of glutamate receptors.3 Subsequent enhancement of Ca2+ influx to intracellular space may induce a Ca2+-dependent enzymatic process,2 resulting in postsynaptic neuronal degeneration.8-10

Neurophysiological studies of the hippocampus in animals after transient ischemia are quite rare.11-18 The perforant path is known to connect monosynaptic projection from the entorhinal cortex to the dentate granule cells.19 and trisynaptically to the CA1 pyramidal cells.20 Ablation of these glutamatergic pathways to the CA1 neurons was found to prevent pathological changes of the CA1 pyramidal cells,21-23 which become apparent several days after transient ischemia.24

To clarify the mechanism of postischemic neuronal degeneration in the hippocampus, I recorded sequential changes of field evoked potentials induced by perforant path stimulation in the dentate gyrus19 and the CA1 region20 of free-moving spontaneously hypertensive rats after transient global ischemia. Functional changes of γ-aminobutyric acid (GABA)-mediated recurrent inhibition in the dentate gyrus were also examined.19,25 Furthermore, I evaluated the protective effects of pretreatment with MK-801 (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-amine, a selective noncompetitive NMDA antagonist.3,26

From the Department of Neurosurgery, Nagasaki University School of Medicine, Nagasaki, Japan.
Address for correspondence: Kazuhiko Suyama, Department of Neurosurgery, Nagasaki University School of Medicine, 7-1 Sakamoto-machi, Nagasaki 852, Japan.
Received March 18, 1991; accepted September 17, 1991.
Materials and Methods

I used fifty-two 12- to 15-week-old spontaneously hypertensive male rats (SHR) weighing 250–300 g in the experiments. In SHR, transient global ischemia consistently results from the four-vessel occlusion method of Pulsinelli et al. Hypotension is not induced because of decreased collateral circulation as compared with that of normotensive rats.

Under intraperitoneal ketamine anesthesia (50 mg/kg), the animals were fixed in a stereotactic frame. Bipolar stimulating electrodes of twisted urethane-coated stainless steel wire 0.2 mm in diameter, uninsulated for approximately 0.5 mm from each tip, were stereotactically placed into the angular bundle (AP: 7.2 mm caudal from the bregma; L: 4.4 mm lateral from the midline; H: 3.6 mm below the cortical surface), according to Pellegrino's atlas. A unipolar recording electrode of the same wire was ipsilaterally inserted into the dentate hilus (AP: 2.5 mm; L: 2.5 mm; H: 3.6 mm) or the CA1 pyramidal cell layer (AP: 2.5 mm; L: 2.5 mm; H: 1.9–2.3 mm), where the maximum field potential evoked by perforant path stimulation was induced. A stainless steel screw, 1.0 mm in diameter, was fixed in the frontal bone as a reference electrode. All the electrodes were connected to a socket and covered with dental cement. A monophasic rectangular pulse with a duration of 0.1 msec and a frequency of 0.3 Hz was applied to the perforant path. Five field potentials amplified by an AC preamplifier with the frequency filter set at 1-5,000 Hz were averaged.

In the dentate gyrus, the typical evoked potential consisted of a short-latency population excitatory postsynaptic potential (EPSP) followed by a population spike (Figure 1A). The initial slope of population EPSP was taken as a measure of population EPSP. Population spike amplitude was determined by measuring the distance from the peak of population spike to a straight line drawn across the base of the spike. In the CA1 region, trisynaptic population spike was recorded following volume-conducted population spike from the dentate gyrus (Figure 1B).

Functional changes of GABA-mediated recurrent inhibition in the dentate gyrus were also examined using paired pulse stimulation to the perforant path. To determine the recovery cycle of population spike, the ratio of test response (PS2) to conditioning response (PS1) was calculated at an interpulse interval of 25, 50, 75, and 100 msec before and on days 1 and 4 after transient ischemia. Before ischemia, the stimulus intensity was set so the amplitude of PS2 reached 25% of PS1 at an interpulse interval of 25 msec (Figure 1C). This level was kept constant throughout the experiment. Furthermore, the relationship between the PS1 and PS2 amplitudes at an interpulse interval of 25 msec was defined using various stimulus intensities as an input/output curve before and on days 1 and 4 after ischemia, since the stimulus threshold needed to induce population spike may change during experimentation. To record population spike in the CA1 region, the stimulus intensity was set at 200% of the threshold intensity and was kept constant throughout the experiment.

Six days after electrode implantation, the vertebral arteries were bilaterally electrocauterized through the transverse foramen during ketamine anesthesia. On the following day, the animals were intubated and mechanically ventilated with 2% halothane in a mixture of 70% nitrous oxide and 30% oxygen. The bilateral carotid arteries were exposed through a midline incision in the neck. The femoral artery was catheterized for blood gas analysis. The temperature of the temporal muscle was continuously monitored and was kept at 37°C during and for 4 hours after ischemia with a heating lamp and pad. After discontinuation of halothane anesthesia, the bilateral carotid arteries were clamped with small metal clips for 20 minutes. The animals were then...
extubated and kept in a light-controlled room. Sequential changes of evoked potentials were recorded before, during, and for 7 days after ischemia (n=7 in both the dentate gyrus and the CA1 region). In eight control animals without ischemia, evoked potentials were also recorded for the same periods (n=4 in each region).

I also examined the effects of MK-801 in the same model. Either 2 mg/kg MK-801 (2 mg/ml in saline; n=5 in each region), 5 mg/kg MK-801 (5 mg/ml in saline; n=5 in each region) or saline (1 ml/kg; n=5 in each region) were intraperitoneally injected 30 minutes before carotid occlusion. Serial changes of evoked potentials were recorded in each group.

Upon completion of all experiments, the animals were deeply anesthetized with pentobarbital and transcardially perfused with saline followed by 10% formalin in pH-adjusted phosphate buffer. Electrode position was confirmed in each animal. Brain coronas were sectioned at a thickness of 10 mm and were stained with toluidine blue for pathological examination. CA1 cell counts were performed using an ocular grid piece (magnification) x 640), along a predetermined distance of three grid lengths (3x470 μm) in the CA1 region (6.0 mm anterior to the interaural line). For each animal, a mean number of CA1 cell counts were calculated from four separate assessments: counts were performed on both the left- and right-hand sides of the brain on two nonconsecutive sections.

For the evoked-potentials study, the mean population spike amplitudes obtained for each time period were evaluated by analysis of variance and Tukey's test for multiple comparisons. For input/output curves, the mean PS2 amplitude obtained for each PS1 amplitude was examined using the above-mentioned test. For pathological examination, the differences between cell counts in the saline-treated and MK-801-treated groups were evaluated using analysis of variance followed by Dunnett's test for comparison of individual MK-801-treated groups with the saline-treated group.

Results

In control animals, I observed no significant change in evoked potentials throughout the experiment. Soon after carotid occlusion, population spike amplitudes in both regions gradually decreased and completely disappeared within 2 minutes, coincident with the disappearance of background electroencephalogram activity. From 5 to 15 minutes after recirculation, population spike with a small amplitude reappeared in both regions. Figure 2 shows the sequential changes of evoked potentials in each region. In the dentate gyrus, population EPSP returned to its preschismic level after 6 hours, then showed transient enhancement from 6 to 24 hours after recirculation. It gradually decreased to 82±8% (mean±SD) of the preschismic control value on day 7 after ischemia. The recovery of population spike was slower than that of population EPSP. The amplitude of population spike returned to its preischemic level 24 hours after recirculation, then gradually decreased to 64±10% (mean±SD) on day 7 after ischemia. In contrary with population spike in the dentate gyrus, complete recovery of the population spike amplitude to its preischemic level was not observed in the CA1 region. At 24 hours after recirculation, the population spike amplitude recovered to only 82±14% of the preischemic level. This was followed by a rapid decrease to 25±15% of the preischemic level on day 2 and complete disappearance on day 7 after ischemia.

Figure 3 shows the input/output curves of the population spike in the dentate gyrus at an interpulse interval of 25 msec. Although there was no significant difference between the mean PS2 amplitudes obtained before ischemia and on day 1 after ischemia, a significant reduction of PS2 was observed on day 4, coincident with a decreased PS1 amplitude. Before ischemia, the PS2 amplitude was markedly inhibited at an interpulse interval of 25 and 50 msec, followed by a facilitatory phase at 75 and 100 msec. Although there was no significant difference between the PS2 amplitudes obtained before ischemia and on day 1 after ischemia, increased inhibition at an interpulse interval of 50 msec and suppression of the facilitation observed at 75 and 100 msec were obvious on day 4 after ischemia (data not shown).

Figure 4 shows the sequential changes of population spike in the dentate gyrus of animals treated with MK-801 or saline. In the group treated with 2 mg/kg MK-801, the population spike change did not differ from that of the saline-treated group except for an enhancement of amplitude at 6 and 12 hours after recirculation. In the group treated with 5 mg/kg MK-801, recovery of the population spike to its preischemic level occurred significantly faster than that of the saline-treated group, and the decrease in the population spike observed in the saline-treated group on days 4 and 7 after ischemia was not seen.
FIGURE 3. Graph of input/output curves of population spike (p-spike) at interpulse interval of 25 msec in dentate gyrus [before ischemia (●), on days 1 (△) and 4 (□) after ischemia in saline-treated group and on day 4 in MK-801 (5 mg/kg)-treated group (○)]. Each point and vertical bar indicates mean amplitude of test response (p-spike 2) and its standard deviation, respectively. Stimulus intensity was set at a level to induce each conditioning response. **p<0.01 on day 4 (□) vs. before ischemia (●) in saline-treated group; *p<0.05 for MK-801 (5 mg/kg)-treated (○) vs. saline-treated (□) groups.

Figure 3 also shows marked enhancement on day 4 of PS2 amplitude in the group treated with 5 mg/kg MK-801 after ischemia except for PS2 when PS1 amplitude was large.

In the CA1 region, recovery of the population spike amplitude was markedly modified in the group treated with 5 mg/kg MK-801 (Figure 5). From 1 to 12 hours after ischemia, population spike amplitudes in the group treated with 5 mg/kg MK-801 were significantly greater than those of the groups treated with 2 mg/kg MK-801 and saline. Furthermore, the decrease in population spike amplitude in the group treated with 5 mg/kg MK-801 was prevented on days 2 and 4 after ischemia and was 53±19% of the preischemic value on day 7.

There were no histological abnormalities in the control animals. Ischemic changes such as condensed nuclei, shrunken cytoplasms, and pyknotic cells were apparent in the CA1 pyramidal cells and the dentate hilus in the saline-treated group on day 7 after ischemia. Other regions of the hippocampus such as the dentate granule cells and the molecular layer of the dentate gyrus did not show marked pathological changes. In the groups treated with MK-801, only the 5 mg/kg dosage partially prevented the ischemic changes of CA1 cells (32% less than control group) (Figure 6).

FIGURE 5. Graph of effects of MK-801 on population spike (PS) in CA1 region. For saline-treated (●), MK-801 (2 mg/kg)–treated (△), and MK-801 (5 mg/kg)–treated (○) groups, each point and vertical bar indicates mean value of PS and its standard deviation, respectively. *p<0.05; **p<0.01 for MK-801 (5 mg/kg)–treated (○) vs. saline-treated (●) groups.

FIGURE 6. Bar graph of total number of normal CA1 pyramidal cells counted along three ocular grid lengths (mean±SD of four counts per animal). ***p<0.01 for MK-801–treated vs. saline-treated groups by Dunnett’s test.
Discussion

In the present study, I evaluated sequential changes of neuronal transmission in the hippocampus of free-moving SHR after transient global ischemia. Neurophysiological studies of the hippocampus in animals after transient ischemia are quite rare. Suzuki et al. reported increased mean firing rates of the CA1 neurons 7–24 hours after transient ischemia in gerbils, an activity that may have been due to excessive release of excitatory amino acids from the presynaptic terminals. However, neurochemical studies using brain microdialysis showed that increased extracellular concentrations of excitatory amino acids returned to preischemic levels within 30 minutes of recirculation. The same hyperexcitability of CA1 neurons was also observed in rats and 3 days after transient ischemia by Chang et al. However, a slowly developing intrinsic pathology of CA1 neurons, rather than increased neuronal transmission from CA3 to CA1, was implicated in this process because the spontaneous firing rate of the CA3 neurons was decreased and the population spike amplitude in the CA1 region evoked by stimulation of the CA3 region was attenuated during the same period of time. I also failed to detect an increased population spike amplitude induced by perforant path stimulation in the dentate gyrus or the CA1 region. In addition, I did not observe electroclinical convulsion throughout the experiment. Buzsáki et al. reported the same results.

I observed a discrepancy in the recovery pattern of population EPSP and population spike in the dentate gyrus during the early posts ischemic period (Figure 2). This finding suggests that failure to generate a spike may be due to membrane dysfunction of the dentate granule cells despite recovered neuronal transmission that could generate a large population EPSP within 24 hours after recirculation.

In this study, the sequential changes of population spike in the dentate gyrus after transient ischemia were quite different from those in the CA1 region (Figure 2). Population spike amplitude in the dentate gyrus recovered 24 hours after recirculation, followed by a gradual decrease to 64 ± 10% of the preischemic level on day 7 after ischemia. At this time, no distinct pathological changes in the dentate granule cells were observed. Onodera et al. reported a reduction of NMDA receptors in both the molecular layer of the dentate granule cells and the CA1 region on day 7 after transient ischemia. These changes may contribute to decreased population spike amplitude in the dentate gyrus in addition to posts ischemic dysfunction of the perforant path. On the other hand, the population spike in the CA1 region showed only partial recovery, followed by a rapid decrease on day 2 after ischemia and disappearance on day 7. These findings were compatible with pathological changes in the CA1 pyramidal cells (Figure 6). The results suggest that a persistent neuronal dysfunction in the CA1 region may develop during the early posts ischemic period. Recent studies on Ca2+ conductance of the postsynaptic membrane and function of protein synthesis in the CA1 region have also shown that persistent failures in neuronal function have already occurred either during transient ischemia or in the early posts ischemic period.

Controversy exists regarding functional changes of the interneuronal inhibitory system of the hippocampus after transient ischemia. In the present study, our results showed an enhancement of recurrent inhibition that coincided with decreased glutamatergic transmission observed as attenuation of population spike amplitude in the dentate gyrus on day 4 after ischemia. Chang et al. obtained similar results in rats under pentobarbital anesthesia on day 2 after transient ischemia. They also showed that paired-pulse inhibition in the CA1 region was not modified on day 2 after ischemia, whereas monosynaptically induced population spike in the CA1 region displayed a significant reduction in amplitude. These results suggest that hyperactivation of the CA1 neurons may not depend on disinhibition of the inhibitory interneuronal circuits. Several mechanisms, such as increased receptivity of interneurons, increased output of interneurons, and increased receptivity of dentate granule cells to GABA, may be involved in the enhancement of recurrent inhibition. Contrary to my results, Buzsáki et al. reported reduction of paired-pulse inhibition in the dentate gyrus on day 5 after ischemia and suggested as the cause malfunctioning of recurrent inhibition that was compatible with the substantial loss of somatostatin-containing neurons in the dentate hilus. However, a decrease in population spike amplitude was also seen. The cause of this discrepancy between results is unknown; however, severity of the ischemia, difference in species, and timing of the evoked potentials study may account for the observed differences.

A noncompetitive NMDA receptor antagonist, MK-801 has been reported to protect against pathological changes of the CA1 pyramidal cells after transient ischemia. However, this protective effect depends on the severity of the induced ischemia. In hippocampal slices, population spike amplitude and paired pulse inhibition in the CA1 region were not impaired, and the long-term potentiation that indicates synaptic plasticity was also preserved 4 days after transient ischemia in gerbils pretreated with 10 mg/kg MK-801. In my experiment, pretreatment with MK-801 facilitated the recovery of the population spike amplitude and prevented the rapid reduction of amplitude in the CA1 region that was characteristic on day 2 after transient ischemia in animals not treated with MK-801. The CA1 neurons were partially reserved on day 7 after ischemia in the group treated with 5 mg/kg MK-801 (Figure 6). In the dentate gyrus, population spike and paired-pulse inhibition were not modified after they returned to their preischemic states (Figures 3 and 4).

In an in vitro study, Balestrino et al. reported that the duration of anoxic depolarization may be critical for the recovery of neuronal transmission. They
showed a significant difference between dentate granule cells and CA1 pyramidal cells regarding the latency of synaptic failure and the magnitude of spreading depression-like depolarization induced by anoxia. Rader and Lanthorn showed that pretreatment with MK-801 delayed the onset of terminal depolarization in hippocampal slices and prevented persistent depolarization that may depend on extracellular Ca²⁺ concentration. These results lead me to believe that MK-801 may protect the CA1 neurons from postischemic dysfunction through inactivation of NMDA receptors during and after transient ischemia, when the extracellular concentration of excitatory amino acids has already increased. Therefore, the prognosis of the CA1 neurons after transient ischemia would be determined during the early postischemic period and would not depend on the postischemic excitotoxic effect of the glutamatergic pathway on the CA1 neurons.

Acknowledgments
I am deeply indebted to Dr. Kazuo Mori, Professor of the Department of Neurosurgery, and to Dr. Kenji Ono, Associate Professor of the Department of Physiology, Nagasaki University School of Medicine, for their excellent advice. I also thank Dr. Hiroshi Baba, Dr. Makio Kaminogo, Dr. Masaki Kurihara, and Dr. Teruaki Kawano of the Department of Neurosurgery, Nagasaki University School of Medicine, for their valuable criticism and suggestions.

References
1. MacDermott AB, Dale N: Receptor, ion channels and synaptic potentials underlying the integrative actions of excitatory amino acid. Trends Neurosci 1987;10:280-284
10. Novelli A, Reilly JA, Lysko PG, Henneberry RC: Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. Brain Res 1988;451:205-211
24. Kirino T: Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res 1982;239:57-69


---

**KEY WORDS** • rats • cerebral ischemia • hippocampus • MK-801
Changes of neuronal transmission in the hippocampus after transient ischemia in spontaneously hypertensive rats and the protective effects of MK-801.

K Suyama

Stroke. 1992;23:260-266
doi: 10.1161/01.STR.23.2.260

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/23/2/260

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/