Hippocampal Unit Activity After Transient Cerebral Ischemia in Rats

Han Soo Chang, MD, Tomio Sasaki, MD, DMSc, and Neal F. Kassell, MD

Single unit activity of CA1 and CA3 neurons in the hippocampus was recorded in rats 1, 2, or 3 days after 10 minutes of transient cerebral ischemia induced by the clamping of both carotid arteries combined with hypotension. In addition, paired pulse inhibition/facilitation of the CA1 population spike was examined on Day 2 using two successive stimuli of the contralateral CA3 region delivered at various intervals. On Day 1, the mean±SEM firing rate in the CA1 region was 0.91±0.42/sec (n=5), which was not significantly different from the control value of 0.98±0.26/sec (n=5). Firing rate increased on Days 2 and 3 to 3.96±0.69/sec (n=5), and 6.49±0.89/sec (n=5), respectively. In the CA3 region, the mean±SEM firing rate of 1.18±0.27/sec in the five control rats sharply dropped to 0.14±0.11/sec in the five Day 1 rats and gradually increased to 0.45±0.11/sec in the five Day 3 rats. Histologic examination of these rats revealed ischemic changes restricted to CA1 neurons on Days 2 and 3. The paired-pulse experiment showed no significant difference between six control and six Day 2 rats in the inhibition of the second population spike with interstimulus intervals of <400 msec. At interstimulus intervals of >500 msec there was facilitation of the second spike, which lasted 5 seconds in Day 2 rats. This facilitation was not observed in control rats. Because CA3 neurons constitute the main input to CA1 pyramidal cells, decreased activity of CA3 neurons indicates less excitatory input to CA1 neurons. The discrepancy in firing rate between CA1 and CA3 neurons suggests that this increased firing rate of CA1 neurons is not caused by increased excitatory input, but by some mechanism intrinsic to the CA1 neuron itself. The fact that paired-pulse inhibition was comparable in control and ischemic rats indicates that inhibitory circuits in the CA1 region are still intact on Day 2. (Stroke 1989;20:1051-1058)

After a brief period of cerebral ischemia, neurons in the CA1 region of the hippocampus are selectively damaged. It takes several days for this neuronal damage to become apparent at the light microscopic level, and this phenomenon therefore has been called delayed neuronal death or the maturation phenomenon. It has been suggested that delayed neuronal death is mediated by the following successive events: 1) excessive release of glutamate from nerve terminals during the ischemic period, 2) activation of N-methyl-D-aspartate (NMDA)-type glutamate receptors, and 3) influx of Ca²⁺ into the neurons, probably through NMDA receptor-linked calcium channels. However, it is not clear what happens in the neurons after these events nor the precise mechanism of the delayed onset of the neuronal damage. In their electrophysiological study, Suzuki et al. reported an increased firing rate in the vulnerable CA1 neurons 7–24 hours after transient cerebral ischemia in gerbils. There are several possible explanations for this hyperexcitability: 1) because of some metabolic or other intrinsic disorder, the neurons can no longer maintain their normal resting membrane potential; 2) there is increased excitatory synaptic input to these neurons, which consequently drives the neurons to the high firing rate; or 3) the inhibitory interneurons in the CA1 region of the hippocampus are impaired, which causes excessive firing of CA1 neurons in response to a normal synaptic input. We recorded the spontaneous activity of CA1 neurons of rats after transient cerebral ischemia to determine whether there were increases in neuronal activity similar to those observed in the gerbil model. At the same time, we also recorded from CA3 neurons, which supply the main input to the CA1 region, to assess the excitatory synaptic drive to the CA1 region. We further investigated the excitability of CA1 neurons and the viability of inhibitory circuits using paired pulse stimulation of the pathway from the contralateral CA3 region.
Materials and Methods

We used 45 male Wistar rats (body wt 300–400 g, Charles River Laboratories, Inc., Wilmington, Massachusetts). Ten minutes of transient cerebral ischemia was induced with the method of two-vessel occlusion with hypotension. The rats were fasted for 24 hours before surgery. After the initial induction of anesthesia, the rats were intubated and mechanically ventilated with a 0.8% halothane–100% oxygen mixture. The electroencephalogram (EEG) was monitored by subcutaneous needle electrodes. The femoral artery and vein were catheterized, and 1 mg/kg pancuronium bromide was injected intravenously for immobilization. Rectal temperature was monitored and kept at 37.0°C using a heating lamp. Through a midline skin incision, both common carotid arteries were exposed and dissected from the vagus nerves. An arterial blood sample was taken for blood gas and glucose analysis, and 50 units of heparin was injected intravenously before induction of ischemia. After discontinuation of halothane, both carotid arteries were clamped using small metal clips and the mean arterial blood pressure was reduced to approximately 50 mm Hg by withdrawing blood from the arterial catheter and was kept at approximately that value throughout the ischemic period by infusing or withdrawing blood through the venous or arterial catheter. Isoelectric EEG recording was confirmed. After 10 minutes, the clips were removed from the carotid arteries and the blood was reinfused through the arterial catheter. The rats were allowed to recover from surgery.

For unit activity analysis, 20 rats, either controls or 1, 2, or 3 days after ischemia, were anesthetized with 50 mg/kg i.p. pentobarbital and placed in a stereotactic frame. Small burr holes were made in the skull for the insertion of electrodes. A Parylene-coated tungsten electrode (Micro Probe Inc., Clarksburg, Maryland) with a tip exposure of 10 μm (impedance 15 MΩ) was used for recording spontaneous unit activity from CA1 or CA3 neurons. Twisted bipolar stimulating electrodes of insulated stainless steel (diameter 100 μm) were placed in the ipsilateral angular bundle (bregma −8.1 mm, lateral 4.4 mm, depth 3.0 mm) and in the contralateral CA3 region (bregma −4.0 mm, lateral 4.0 mm, depth 3.5 mm). The stereotactic coordinates for recording were bregma −4.0 mm, lateral 2.0 mm for the CA1 region and bregma −4.0 mm, lateral 4.0 mm for the CA3 region. The positions of the stimulating electrodes were adjusted for maximal response. For CA1 recording, unit activity was encountered at approximately 2 mm from the surface, and the correct positioning of the electrode tip in the pyramidal cell layer was further confirmed by the laminar profile of the field potentials evoked by the two electrodes. For CA3 recording, as the electrode was lowered the first unit activity of the CA1 or CA2 cell layer was encountered at approximately 2.5–3 mm from the surface, and past that cell layer the unit activity of CA3 neurons could usually be obtained at approximately 0.5–1 mm below the first cell layer. The positioning of the electrode tip in the CA3 cell layer was further confirmed by the laminar profile of the evoked responses and especially by the antidromic population spike evoked by stimulation of the contralateral CA3 region. Single units were isolated by careful adjustment of electrode position, and those units with good isolation (with spike amplitude >1.5 times that of the second largest spike) were sampled. The signal was amplified, and the unit activity was transformed to 5-volt pulses by a window discriminator (Model 120, WPI, New Haven, Connecticut). The output of the window discriminator was transferred to a personal computer, where each unit’s activity was analyzed for 2.5 minutes and the average firing rate was calculated.

Twelve rats were prepared for paired pulse analysis (six control and six 2 days after ischemia) as described above. A recording electrode was positioned in the pyramidal cell layer of the CA1 region. Two successive stimuli were delivered through a contralateral CA3 stimulating electrode, with interstimulus intervals of 50, 100, 200, 300, 400, 500, 600, 700, 800, and 900 msec and 1, 2, 3, 4, and 5 seconds. Stimulation consisted of a rectangular pulse with a width of 300 msec. Supramaximal intensity was used, giving a maximal population excitatory postsynaptic potential (EPSP) and a population spike. The ratio of the amplitude of the second population spike (P2) to the first population spike (P1) was calculated for each interstimulus interval. Each response was captured on an oscilloscope screen (5111A Storage Oscilloscope, Tektronix, Beaverton, Oregon) and recorded on Polaroid film (Cambridge, Massachusetts). The amplitudes of P1 and P2 were measured and the P2:P1 ratio was calculated for each interstimulus interval.

All 32 rats that underwent the physiology were deeply anesthetized with pentobarbital and were perfused transcardially by 10% formalin in pH-adjusted phosphate buffer. In addition, six Day 4 and seven Day 7 rats were perfused similarly for histological analysis only. Brains were embedded in glycol methacrylate (LKB-Produkter, Bromma, Sweden) and sectioned coronally at a thickness of 1.0 μm. Sections were stained with toluidine blue for light microscopy.

For the physiological analysis, mean firing rates of CA1 or CA3 neurons in control, Day 1, Day 2, and Day 3 rats were analyzed first by single-factor analysis of variance, and then the difference between the control and the other groups was further analyzed using Dunnet’s multiple comparison test. For electrophysiology (paired pulse) data, the P2:P1 ratio for each interstimulus interval was analyzed separately using the Mann-Whitney U test.
TABLE 1. Firing Rate of CA1 and CA3 Neurons in Rats After 10 Minutes of Transient Cerebral Ischemia

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats</th>
<th>No. of cells</th>
<th>Firing rate (sec⁻¹)</th>
<th>No. of rats</th>
<th>No. of cells</th>
<th>Firing rate (sec⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>22</td>
<td>0.98±0.26</td>
<td>5</td>
<td>21</td>
<td>1.18±0.27</td>
</tr>
<tr>
<td>Day 1</td>
<td>5</td>
<td>20</td>
<td>0.91±0.42</td>
<td>5</td>
<td>20</td>
<td>0.14±0.04*</td>
</tr>
<tr>
<td>Day 2</td>
<td>5</td>
<td>28</td>
<td>3.96±0.69*</td>
<td>5</td>
<td>18</td>
<td>0.34±0.07*</td>
</tr>
<tr>
<td>Day 3</td>
<td>4</td>
<td>36</td>
<td>6.49±0.89*</td>
<td>5</td>
<td>22</td>
<td>0.45±0.11*</td>
</tr>
</tbody>
</table>

Firing rate, mean±SEM.
*<p<0.01, significantly different from control by Dunnet's multiple comparison test.

Results

In unit activity analysis, results of 187 units recorded from 20 rats (five for each group) are summarized in Table 1 and Figure 1. The mean±SEM firing rate of CA1 neurons in control rats is consistent with previous reports.¹⁵,¹⁶ There was no significant difference between the firing rates of CA1 and CA3 neurons in control rats. In the CA1, the firing rate was not significantly different from control on Day 1 but was significantly increased on Days 2 and 3. However, in the CA3 region the result was markedly different; the firing rate decreased sharply on Day 1 and then gradually increased on Days 2 and 3.

The paired-pulse results obtained from six control and six Day 2 rats are shown in Figure 2. The maximum P1 amplitude tended to be smaller, although not significantly so, in Day 2 than in control rats, whereas the maximum amplitude of the population EPSP was not so different between groups (Table 2). With shorter interstimulus intervals, there was a marked inhibition of P2 in both control and Day 2 rats. This inhibition lasted for approximately 300 msec, and the rate of decay was very similar between groups. However, after 400 msec, there was a discrepancy between groups. In control rats, the P2/P1 ratio was close to 1.0 throughout the rest of the period, whereas in Day 2
FIGURE 2. Top: Superimposed traces of field potentials obtained in CA1 pyramidal cell layer of rats evoked by two successive stimulations of contralateral CA3 electrode with various interstimulus intervals (200 ms, 500 ms, and 1 second) after 10 minutes of transient cerebral ischemia. Solid lines, first responses; dashed lines, second responses. Bottom: Comparison of mean±SEM paired pulse inhibition/facilitation curves in control (○, n=6) and Day 2 (●, n=6) rats. Ratio (P2/P1) was plotted against various interstimulus intervals (ISI). *p<0.05, **p<0.01, different from control by Mann-Whitney U test.

rats P2 was markedly facilitated, and this facilitation was present even with the interstimulus interval of 5 seconds.

In Day 1 rats, CA1 neurons did not show marked histologic abnormalities by light microscopy. However, in Day 2 and 3 rats, ischemic changes were apparent in the CA1 region. These changes included condensed nuclei, shrunken cytoplasm, and occasional pyknotic neurons (Figure 3) and were restricted to the CA1 neurons. Other regions of the hippocampus, such as the CA3 region and the dentate gyrus, did not show marked pathologic changes. In six of seven rats that were subjected to histology on Day 7 without the electrophysiological procedure, there was an almost complete bilateral destruction of the CA1 region (Figure 3).

Discussion

Despite a number of pioneering studies, the precise mechanism of delayed neuronal death after transient cerebral ischemia is still unknown. It seems well established that the excitatory synaptic input to the CA1 neurons and the activation of NMDA receptors play an important role in causing delayed neuronal death. However, it is not clear whether this "excitotoxic" mechanism is also present during the chronic stage of transient cerebral ischemia or whether it is active only during
the acute stage. Several studies suggest that mechanisms intrinsic to the CA1 neuron itself, such as the impairment of protein synthesis in the vulnerable neurons, are involved during the chronic stage, but other studies indicate involvement of the neuronal circuitry of the hippocampus during this period. Johansen et al found that the number of somatostatin-containing neurons decreases in the dentate hilus 2 days after transient ischemia in rats, and the authors raised the possibility that decreased inhibition of dentate granule cells causes delayed damage to CA1 neurons. Prolonged seizure causes damage to CA1 neurons, and even tetanic stimulation of the entorhinal cortex can produce CA1 damage. Therefore, it is possible that increased excitation of the hippocampal circuitry takes place during the chronic stage after transient ischemia and damages the CA1 neurons. In addition, although the inhibitory interneurons are reportedly resistant to transient cerebral ischemia, it is still theoretically possible that some functional impairment of these interneurons can cause hyperexcitability of CA1 neurons.

In our study, neurons in the CA1 region showed marked increases in firing rate 2 and 3 days after 10 minutes of transient cerebral ischemia. On the other hand, the firing rate decreased during the same period in the CA3 region. Since CA3 neurons constitute the main input to CA1 neurons, the decreased firing rate of CA3 neurons indicates a decreased excitatory input to the CA1 region from the CA3 region. These results indicate that there is no increase in activity over the trisynaptic circuit from the entorhinal cortex to the CA1 region via the dentate gyrus and the CA3 region. It remains possible, although unlikely, that the direct input from the entorhinal cortex to the CA1 region is hyperactive. Therefore, this discrepancy in firing rate between the CA1 and CA3 regions indicates that the increased firing rate of CA1 neurons is not caused by an increased excitatory input, but by some mechanism intrinsic to the CA1 neuron itself. As shown in Figure 1, the firing rate of CA1 neurons increased in a somewhat linear fashion from Day 1 to Day 3, and this suggests a progressive nature of the underlying mechanism.

There is some difference between our results and those of Suzuki et al on spontaneous firing of CA1 neurons. Those authors reported that the mean±SD firing rate of CA1 neurons was 9.2±4.7/sec in control gerbils and increased to 29.1±9.8/sec 1 day after ischemia, whereas our results were 0.98±0.26/sec (mean±SEM) in control rats and 6.49±0.89/sec 3 days after ischemia. These authors probably reported larger values because they measured the maximum instead of the average frequency during the recording period. Another difference is that they found increased firing rates 7 hours to 1 day after transient cerebral ischemia and could not detect neuronal activity on Day 2, whereas we found that the firing rate started to increase on Day 2 and could still be recorded on Day 3. This difference in time course can probably be explained by the difference in animal models, in which the severity of ischemia could well be different. As Klatzo stated, varying the severity of ischemia can alter the time course of delayed neuronal death.

On the other hand, the time course of our histologic study closely resembled that of Kirino, who described the initial appearance of pathologic findings on Day 2. In addition, the time course of the delayed disturbance of calcium homeostasis in CA1 neurons, described by Dux et al and Sakamoto et al, is similar to that of the increased activity we observed. It is possible that these findings represent the same underlying pathologic process in the CA1 neurons.

Although we did not evaluate neuronal activity after Day 4, our histologic study showed that there was extensive damage to CA1 neurons in three of six rats on Day 4 and in six of seven rats on Day 7. Furthermore, in one of five rats studied physiologically on Day 3, we could not detect any unit activity or obtain evoked potentials in the CA1 region, and it was generally more difficult to obtain a unit on Day 3 than on Days 1 or 2. Therefore, we believe that in our experimental condition, most CA1 neurons died during Days 4–6. Because our histologic examination revealed that most CA1 neurons were still alive on Day 3, the population of neurons that we recorded electrophysiologically on Day 2 represent the CA1 pyramidal cells dying or destined to die later.

The paired-pulse experiment clearly showed that there was no difference in the inhibition of P2, at least at short interstimulus intervals. According to previous studies, the earlier part of this inhibition is probably mediated by γ-amino-n-butyric acid (GABA)-ergic interneurons affecting the GABA receptors in the postsynaptic neurons, and the later part is probably mediated by activation of calcium-induced potassium conductance. In addition to these, Gustafsson and Wingström showed that a long-lasting hyperpolarization of CA1 pyramidal cells after tetanic stimulation is mediated by activation of an electrogenic sodium pump in the postsynaptic CA1 neurons. Thus, our results indicate that the GABA-mediated inhibition caused by interneuron circuits remains intact on Day 2 after transient cerebral ischemia, when the activity of CA1 neurons is already increased.
Figure 3. Light micrographs of CA1 pyramidal cell layers; 1-μm sections of glycol methacrylate-embedded tissue, toluidine blue staining, x 1480. Top: control rats. Middle: 2 days after 10 minutes of transient cerebral ischemia. Bottom: 7 days after 10 minutes of transient cerebral ischemia.
As to the delayed facilitation of P2, which was observed after 400 msec and lasted until 5 seconds, interpretation is difficult. Even in control rats, this kind of delayed facilitation was observed when they were tested by weaker stimulus intensity with smaller population spikes (data not shown). And, as shown in Table 2, the population spike was smaller, although not significantly so, in ischemic rats than in control rats, whereas the amplitude of population EPSP was not so different. Therefore, the delayed facilitation we observed might merely reflect the possibility that, for some unknown reason, the population spike could not reach the saturation value in Day 2 rats.

We conclude that 1) the spontaneous firing rate of CA1 neurons in the rat hippocampus is increased 2 and 3 days after transient cerebral ischemia while that of CA3 neurons is decreased during this period; 2) since CA3 neurons constitute the main excitatory input to the CA1 region, this increased firing rate of CA1 neurons is not caused by increased excitatory drive; 3) the recurrent inhibitory circuit in the CA1 region is still intact 2 days after ischemia, excluding the possibility that dysfunction of the interneurons in the CA1 region causes the increased CA1 firing rate; and 4) some slowly developing pathologic process intrinsic to the CA1 region that is triggered by events during the acute stage of ischemia could cause the increased firing rate of CA1 neurons and their eventual death. Our results were obtained from rats during the chronic stage after transient cerebral ischemia and do not necessarily contradict other studies that show the importance of excitatory input to the CA1 region during the acute stage of ischemia.

Acknowledgments

We thank Sarah B. Hudson, Grace I. Asban, and Thomas P. Harrison for excellent technical assistance. We also thank Oswald Steward, PhD, and Richard A. Tomasulo, MD, for helpful discussions and Lucille Staiger for preparation of the manuscript.

References

1. Kirino T: Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res 1982;239:57–69


KEY WORDS • cerebral ischemia • hippocampus • rats
Hippocampal unit activity after transient cerebral ischemia in rats.
H S Chang, T Sasaki and N F Kassell

Stroke. 1989;20:1051-1058
doi: 10.1161/01.STR.20.8.1051

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/20/8/1051