Transient Neurophysiological Changes in CA3 Neurons and Dentate Granule Cells After Severe Forebrain Ischemia In Vivo

T. M. GAO,¹ E. M. HOWARD,² AND Z. C. XU²

¹Department of Physiology, The First Military Medical University, Guangzhou 510515, People’s Republic of China; and ²Department of Neurology, University of Tennessee at Memphis, Memphis, Tennessee 38163

Gao, T. M., E. M. Howard, and Z. C. Xu. Transient neurophysiological changes in CA3 neurons and dentate granule cells after severe forebrain ischemia in vivo. J. Neurophysiol. 80: 2860–2869, 1998. The spontaneous activities, evoked synaptic responses, and membrane properties of CA3 pyramidal neurons and dentate granule cells in rat hippocampus were compared before ischemia and ≤7 days after reperfusion with intracellular recording and staining techniques in vivo. A four-vessel occlusion method was used to induce ~14 min of ischemic depolarization. No significant change in spontaneous firing rate was observed in both cell types after reperfusion. The amplitude and slope of excitatory postsynaptic potentials (EPSPs) in CA3 neurons decreased to 50% of control values during the first 12 h reperfusion and returned to preischemic levels 24 h after reperfusion. The amplitude and slope of EPSPs in granule cells slightly decreased 24–36 h after reperfusion. The duration of spike width of CA3 and granule cells became longer than that of control values during the first 12 h reperfusion. The spike threshold of both cell types significantly increased 24–36 h after reperfusion, whereas the frequency of repetitive firing evoked by depolarizing current pulse was decreased during this period. No significant change in rheobase and input resistance was observed in CA3 neurons. A transient increase in rheobase and a transient decrease in input resistance were detected in granule cells 24–36 h after reperfusion. The amplitude of fast afterhyperpolarization in both cell types increased for 2 days after ischemia and returned to normal values 7 days after reperfusion. The results from this study indicate that the neuronal excitability and synaptic transmission in CA3 and granule cells are transiently suppressed after severe forebrain ischemia. The depression of synaptic transmission and neuronal excitability may provide protection for neurons after ischemic insult.

INTRODUCTION

The vulnerability of neurons to cerebral ischemia varies widely among different regions in the CNS (Graham 1992; Pulvinelli 1985). Different neuronal populations within a brain region also show different susceptibility to ischemic insult. Studies have shown that CA1 pyramidal neurons in hippocampus die 3–7 days after transient forebrain ischemia, whereas CA3 neurons and dentate granule cells in the same region remain viable (Kirino 1982; Pulvinelli et al. 1982). The mechanisms of this selective neuronal damage are not fully understood. Glutamate excitotoxicity was suggested as a potential mediator of postischemic cell injury (Choi and Rothman 1990; Rothman and Olney 1986). However, the electrophysiological evidence for hyperactivity in hippocampus after ischemia remains controversial. By using extracellular recording technique, some studies showed an increase (Chang et al. 1989; Suzuki et al. 1983), whereas others showed a decrease (Buzsaki et al. 1989; Furukawa et al. 1990; Imon et al. 1991) in neuronal firing rate in CA1 area after ischemia. Recently, it has been shown in an in vivo preparation that the spontaneous firing rate of CA1 pyramidal neurons reduced after severe forebrain ischemia, and the excitability in most of the CA1 neurons progressively decreased (Gao et al. 1998a,b). In contrast, the synaptic transmission in neurons with decreased excitability was enhanced for >12 h after reperfusion (Gao and Xu 1996; Gao et al. 1998a). Because most of the CA1 neurons will die after such severe ischemia (Ren and Xu 1995), these electrophysiological changes were closely associated with the mechanisms of postischemic neuronal injury. On the other hand, no enhancement in synaptic transmission was observed in CA1 neurons after a 5-min sublethal ischemia; the firing rate and neuronal excitability only transiently depressed in these neurons (Xu and Pulvinelli 1994, 1996). The results from these studies suggested that the enhancement of synaptic transmission rather than the increase of neuronal firing rate may play an important role in the pathogenesis of postischemic cell death.

If the electrophysiological changes observed in CA1 neurons after severe ischemia are indeed associated with mechanisms of postischemic cell death, the electrophysiological responses of ischemia-resistant neurons, such as CA3 pyramidal neurons and dentate granule cells, should be different. An extracellular recording study in vivo showed that the activity of CA1 neurons in rat hippocampus was enhanced, whereas the activity of CA3 neurons was suppressed after transient ischemia (Chang et al. 1989). Other studies with in vitro preparation have shown that polysynaptic synchronized bursts were readily evoked in CA3 neurons after brief anoxic episodes (Ben-Ari and Cherubini 1988). The excitatory postsynaptic potentials (EPSPs) in CA3 neurons or granule cells were more resistant to hypoxia or aglycemia than those in CA1 region (Aitken and Schiff 1986; Balestri et al. 1989; Cherubini et al. 1989; Crepel et al. 1992; Kass and Lipton 1986). However, little information is available concerning whether these neurophysiological differences between CA1 and CA3 neurons after ischemia are related to the susceptibility of these neurons.
To distinguish whether the electrophysiological changes in CA1 neurons after severe ischemia are associated with mechanisms of posts ischemic cell death or just the common responses to ischemic stress, it is important to reveal the electrophysiological changes in ischemia-resistant neurons under the same severe ischemic condition. To address this question, we examined the spontaneous activities, evoked synaptic potentials, and membrane properties of CA3 pyramidal neurons and dentate granule cells after severe transient ischemia with intracellular recording and staining techniques in vivo. A preliminary report of this study appeared in abstract form (Gao and Xu 1997).

METHODS

Transient forebrain ischemia

Male adult Wistar rats (250–350 g, Charles River) were used. Transient forebrain ischemia was induced with the four-vessel occlusion method (Pulsinelli and Brierley 1979) with some modifications. The animals were fasted overnight to provide uniform blood glucose levels. For surgical preparation the animals were anesthetized with 1–2% halothane mixed with 33% O2-66% N2. The arterial Po2 was maintained at ~120 mmHg, and the PCO2 was maintained at ~40 mmHg. An occluding device (a loop of silicone tubing) was placed loosely around each carotid artery. The animal was then placed on a stereotaxic frame, and the core body temperature was maintained at 37°C through a temperature control unit (TC-120, Medical System). The vertebral arteries were electrocauterized. A small temperature probe (0.8 mm OD) was inserted beneath the skull in the extradural space after which brain temperature was maintained at 37°C with a heating lamp. Severe forebrain ischemia was induced by occluding both common carotid arteries to induce ischemic depolarization for ~14 min.

Recording of ischemic depolarization

Extracellular DC potential in CA1 region was monitored during ischemia. The recording microelectrodes were pulled from glass capillaries with filaments (A-M system) to a tip resistance of ~5 M (when filled with 2 M NaCl). A burr hole was drilled at 4.0 mm anterior to interaural line, 3.0 mm lateral from the midline. A microelectrode was inserted into the CA1 region of hippocampus (~2.5 mm below brain surface). After the baseline recording, forebrain ischemia was initiated by tightening the occluding device. Ischemic depolarization as indicated by DC potential shifting from 0 to ~20 mV occurred ~2.5 min after occlusion. Occlusion of carotid arteries was terminated at 12 min after the onset of ischemic depolarization. On releasing the common carotid arteries, cerebral blood flow resumed immediately, and the DC potential gradually returned to 0 mV at ~2 min. The duration of ischemic depolarization was measured from the onset of negative deflection of DC potential to the point at which DC potential completely returned to preischemic levels after recirculation. Only the animals with ID of ~14 min were used for electrophysiological experiments. For recordings >12 h after reperfusion, animals were returned to the cage after reperfusion and allowed free access to water and food. The animals were then reanesthetized and prepared for recording at different time points after reperfusion.

Intracellular recording and staining

Preparation for intracellular recording and staining in vivo was performed as previously described (Xu 1995; Xu and Pulsinelli 1996). Briefly, under 1–2% halothane anesthesia, the skull was opened to expose the recording site and for placement of stimulus electrodes. A pair of bipolar stimulating electrodes was placed into the ipsilateral perforant pathway at a 25° angle to the vertical (AP 1.5 mm, ML 1.5–2.5 mm, DV 4.0 mm) and was used to deliver constant current pulses of 0.1-ms duration at various intensities. Recording electrodes were pulled from glass capillaries with filaments to a tip resistance of 50–80 MΩ when filled with a solution of 3% neurobiotin (Vector) in 2 M potassium acetate. Cerebral spinal fluid was drained via a cisternal puncture to reduce brain pulsation. The animal was suspended by a clamp applied to the tail. After placement of a microelectrode in the cortex above the hippocampus for recording, the exposed surface of the brain was covered with soft paraffin wax. After impalement, the neurons with stable membrane potential of at least ~50 mV were selected for further study. Data were digitized and stored with Macintosh computer with data acquisition program Axodata (Axon Instruments). Analysis of variance (ANOVA, Fisher’s PLSD) was used for statistical analysis (Statview, Abacus Concepts).

RESULTS

We used 62 animals, among which 19 were control animals and the rest were subjected to forebrain ischemia that resulted in ischemic depolarization of 13.9 ± 0.6 min (mean ± SD). The ischemia of such severity and duration consistently produced a widespread cell death in CA1 pyramidal neurons of rat hippocampus (Ren and Xu 1995). Figure 1 is an example of hippocampus 7 days after ischemia. All CA1 pyramidal neurons died in this animal, but the CA3 neurons and granule cells remained intact. An intracellularly stained CA3 pyramidal neuron was evident in this section. A total of 136 neurons were successfully stained after recording; 69 were identified as CA3 pyramidal neurons, and 67 were granule cells. No degenerating signs were observed in these neurons at any time points after reperfusion. Examples of intracellularly stained CA3 and granule cells are presented in Fig. 2. On the basis of stereotaxic parameters and electrophysiological characteristics of each cell type, 19 unidentified neurons were considered CA3 cells, and 12 were considered granule cells and were included in this study. To investigate the time course of electrophysiological alterations after ischemia, neurons were grouped according to the time after reperfusion in which they were recorded: 0–12 h (median: 6 h for CA3 cells, 4 h for granule cells); 12–24 h (median: 21 h for CA3 cells, 19 h for granule cells); 24–36 h (median: 29 h for CA3 cells, 31 h for granule cells); 36–48 h (median: 43 h for CA3 cells, 44 h for granule cells); and 7 days.

Spontaneous activity and evoked potentials after ischemia

Compared with preischemic value, the spontaneous firing rate of CA3 neurons slightly decreased within 24-h reperfusion...
FIG. 1. Photomicrographs showing hippocampal region 7 days after ~14 min ischemic depolarization. This is a coronal section counterstained with hematoxylin and eosin. A wide spread neuronal death was apparent in the CA1 region as shown in the insert at the top right corner with higher magnification. The pyramidal neurons in the CA3 region and the granule cells in the dentate gyrus were intact. A CA3 pyramidal neuron was intracellularly stained after recording (arrow).

and that of granule cells also decreased 24–36 h after reperfusion. No statistical significance was detected in these changes. The spontaneous firing rate of both cell types returned to control levels 48 h after reperfusion (Tables 1 and 2).

The postsynaptic potentials were evoked from CA3 and granule cells by stimulation of ipsilateral perforant pathway at 2.5 times the threshold stimulus intensity. The EPSPs were characterized by their amplitude, slope (measured from 10 to 90% of the maximal amplitude), duration (measured at 50% of maximal amplitude), threshold, and latency. In CA3 neurons, the amplitude of EPSPs decreased to ~50% of control value ($P < 0.01$) shortly after reperfusion but quickly returned to control levels ~21 h after reperfusion (Fig. 3E). The temporal profile of postsischemic change in EPSP slope exhibited a similar pattern as that of EPSP amplitude. The threshold of stimulus intensity for EPSPs in CA3 neurons slightly increased shortly after reperfusion and then reduced to 50% of control value ($P < 0.05$) ~21 h after reperfusion. The threshold for EPSPs returned to preischemic levels at ~29 h after reperfusion (Fig. 3F). No significant difference in the duration and latency of EPSPs was observed in CA3 neurons after reperfusion. The amplitude of inhibitory postsynaptic potentials (IPSPs) in CA3 neurons was $-4.70 \pm 2.10 \text{ mV (} n = 11 \text{) before ischemia and increased to } -6.97 \pm 3.02 \text{ at } 21 \text{ h after reperfusion (} n = 10 \text{). No statistical significance was detected in this change. The amplitude of IPSPs returned to control levels at later time points.}

The amplitude of EPSPs evoked from granule cells slightly reduced 19–31 h after reperfusion and returned to control levels thereafter (Fig. 4E). No statistical significance was detected in these changes. Similar changes in the rising slope of EPSPs

FIG. 2. Examples of an intracellularly stained CA3 pyramidal neuron (A) and dentate granule cell (B). The section was lightly counterstained with cresyl violet.
CA3 AND GRANULE CELLS AFTER ISCHEMIA

TABLE 1. Electrophysiological properties of CA3 neurons after reperfusion

<table>
<thead>
<tr>
<th></th>
<th>RMP, mV</th>
<th>Spike Height, mV</th>
<th>Spike Width, ms</th>
<th>Rm, MΩ</th>
<th>Time Constant, ms</th>
<th>Spontaneous Firing, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before ischemia</td>
<td>−66 ± 5.5</td>
<td>75 ± 9.7</td>
<td>0.76 ± 0.15</td>
<td>26.6 ± 8.5</td>
<td>15.2 ± 7.6</td>
<td>1.28 ± 1.38</td>
</tr>
<tr>
<td>(9)</td>
<td></td>
<td>(22)</td>
<td>(22)</td>
<td>(17)</td>
<td>(17)</td>
<td>(10)</td>
</tr>
<tr>
<td>~6 h after reperfusion</td>
<td>−61 ± 6.1*</td>
<td>78 ± 8.7</td>
<td>0.89 ± 0.26*</td>
<td>20.7 ± 5.0</td>
<td>10.9 ± 4.8</td>
<td>0.85 ± 1.29</td>
</tr>
<tr>
<td>(11)</td>
<td></td>
<td>(14)</td>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
<td>(10)</td>
</tr>
<tr>
<td>~21 h after reperfusion</td>
<td>−64 ± 4.6</td>
<td>77 ± 5.8</td>
<td>0.76 ± 0.07</td>
<td>23.8 ± 1.7</td>
<td>13.2 ± 4.5</td>
<td>0.65 ± 1.31</td>
</tr>
<tr>
<td>(11)</td>
<td></td>
<td>(10)</td>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
<td>(10)</td>
</tr>
<tr>
<td>~29 h after reperfusion</td>
<td>−64 ± 3.2</td>
<td>75 ± 7.9</td>
<td>0.73 ± 0.08</td>
<td>29.0 ± 12.8</td>
<td>17.3 ± 7.4</td>
<td>2.13 ± 3.56</td>
</tr>
<tr>
<td>(12)</td>
<td></td>
<td>(15)</td>
<td>(10)</td>
<td>(10)</td>
<td>(10)</td>
<td>(8)</td>
</tr>
<tr>
<td>~43 h after reperfusion</td>
<td>−66 ± 4.6</td>
<td>76 ± 10.4</td>
<td>0.77 ± 0.11</td>
<td>29.3 ± 10.9</td>
<td>14.3 ± 8.5</td>
<td>1.11 ± 1.82</td>
</tr>
<tr>
<td>(15)</td>
<td></td>
<td>(17)</td>
<td>(16)</td>
<td>(16)</td>
<td>(16)</td>
<td>(10)</td>
</tr>
<tr>
<td>7 d after reperfusion</td>
<td>−65 ± 2.5</td>
<td>76 ± 10.8</td>
<td>0.82 ± 0.09</td>
<td>32.6 ± 7.8</td>
<td>12.1 ± 2.3</td>
<td>1.34 ± 1.01</td>
</tr>
<tr>
<td>(10)</td>
<td></td>
<td>(10)</td>
<td>(8)</td>
<td>(8)</td>
<td>(8)</td>
<td>(7)</td>
</tr>
</tbody>
</table>

Values are means ± SD with number of neurons in parentheses. Spike height is measured from the resting membrane potential. Spike width is measured at one-half of the peak amplitude of the action potential. Input resistance (Rm) is derived from the linear portion of the current-voltage curve (0 to −0.5 nA). Time constant is derived from transients of hyperpolarizing pulse (−0.3 nA, 200 ms). RMP, resting membrane potential. * P < 0.01, analysis of variance.

Membrane property changes after ischemia

The membrane properties of CA3 neurons before and at different periods after ischemia are summarized in Table 1. Within the first 12 h reperfusion, the resting membrane potential of CA3 neurons depolarized from −66 ± 5.5 mV before ischemia to −61 ± 6.1 mV (P < 0.01), and the spike width was increased from 0.76 ± 0.15 to 0.89 ± 0.26 ms (P < 0.01). Both properties returned to preischemic levels at later time points. A transient decrease in input resistance and time constant were also observed in CA3 neurons ~6 h after reperfusion, but no statistical significance was detected. The current-voltage (I-V) relationship of CA3 cells was determined by passing constant current puls (200 ms, −1.0 to 0.5 nA, 0.1 nA/step) and measured at the steady state of the transients (160–180 ms after pulse onset) from the averages of four recordings. As shown in Fig. 5, no significant difference was observed in I-V relationship at different intervals after reperfusion except the transient reduction of I-V curve slope ~6 h after reperfusion, indicating a decrease in membrane input resistance during this period.

As presented in Table 2, the resting membrane potential, spike height, and time constant of granule cells remained close to normal ranges after ischemia. However, a transient change in spike width and input resistance of granule cells was detected after reperfusion. The spike width of granule cells decreased from 0.56 mV to 0.38 mV at 21 h after reperfusion (P < 0.01).

TABLE 2. Electrophysiological properties of granule cells after reperfusion

<table>
<thead>
<tr>
<th></th>
<th>RMP, mV</th>
<th>Spike Height, mV</th>
<th>Spike Width, ms</th>
<th>Rm, MΩ</th>
<th>Time Constant, ms</th>
<th>Spontaneous Firing, Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before ischemia</td>
<td>−71 ± 6.2</td>
<td>85 ± 11.5</td>
<td>0.80 ± 0.12</td>
<td>44.2 ± 13.7</td>
<td>9.8 ± 6.7</td>
<td>0.19 ± 0.50</td>
</tr>
<tr>
<td>(15)</td>
<td></td>
<td>(15)</td>
<td>(15)</td>
<td>(11)</td>
<td>(11)</td>
<td>(15)</td>
</tr>
<tr>
<td>~4 h after reperfusion</td>
<td>−77 ± 10.2</td>
<td>89 ± 13.1</td>
<td>0.92 ± 0.16*</td>
<td>46.2 ± 9.3</td>
<td>8.5 ± 2.8</td>
<td>0.12 ± 0.42</td>
</tr>
<tr>
<td>(12)</td>
<td></td>
<td>(13)</td>
<td>(9)</td>
<td>(9)</td>
<td>(9)</td>
<td>(12)</td>
</tr>
<tr>
<td>~19 h after reperfusion</td>
<td>−73 ± 9.6</td>
<td>79 ± 16.7</td>
<td>0.78 ± 0.12</td>
<td>44.0 ± 14.0</td>
<td>14.2 ± 8.8</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>(17)</td>
<td></td>
<td>(16)</td>
<td>(16)</td>
<td>(13)</td>
<td>(13)</td>
<td>(13)</td>
</tr>
<tr>
<td>~31 h after reperfusion</td>
<td>−72 ± 5.3</td>
<td>82 ± 11.8</td>
<td>0.88 ± 0.23</td>
<td>26.3 ± 19.1‡</td>
<td>7.8 ± 3.8</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>(12)</td>
<td></td>
<td>(9)</td>
<td>(9)</td>
<td>(11)</td>
<td>(11)</td>
<td>(9)</td>
</tr>
<tr>
<td>~44 h after reperfusion</td>
<td>−72 ± 6.9</td>
<td>80 ± 7.8</td>
<td>0.85 ± 0.20</td>
<td>35.0 ± 12.8</td>
<td>9.1 ± 6.8</td>
<td>0.20 ± 0.64</td>
</tr>
<tr>
<td>(16)</td>
<td></td>
<td>(19)</td>
<td>(12)</td>
<td>(12)</td>
<td>(12)</td>
<td>(13)</td>
</tr>
<tr>
<td>7 d after reperfusion</td>
<td>−76 ± 6.3</td>
<td>84 ± 7.8</td>
<td>0.83 ± 0.09</td>
<td>44.1 ± 12.4</td>
<td>13.9 ± 5.2</td>
<td>0.14 ± 0.38</td>
</tr>
<tr>
<td>(7)</td>
<td></td>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
<td>(7)</td>
</tr>
</tbody>
</table>

Values are means ± SD with number of neurons in parentheses. Spike height is measured from the resting membrane potential. Spike width is measured at one-half of the peak amplitude of the action potential. Input resistance (Rm) is derived from the linear portion of the current-voltage curve (0 to −0.5 nA). Time constant is derived from transients of hyperpolarizing pulse (−0.3 nA, 200 ms). RMP, resting membrane potential. ‡ P < 0.01, analysis of variance.
cells increased from $0.80 \pm 0.12$ ms before ischemia to $0.92 \pm 0.16$ ms $\sim 4$ h after reperfusion ($P < 0.05$) and then returned to control levels at later time points. The input resistance was $44.2 \pm 13.7$ M$\Omega$ in control animals. This value remained unchanged during the first 24 h after reperfusion but significantly decreased to $26.3$ M$\Omega$ at 31 h after reperfusion ($P < 0.01$) and then gradually recovered to preischemic level. The $I-V$ relationship of granule cells before and after ischemia was about the same except for a significant decrease in the slope of $I-V$ curve at $\sim 31$ h after reperfusion, indicating a transient reduction of input resistance (Fig. 6).

The changes in neuronal excitability of CA3 and granule cells were evaluated by comparing the spike threshold and rheobase before ischemia and at different intervals after reperfusion. The spike threshold was measured at the beginning of the upstroke of the action potential, and the rheobase was determined as the minimal intensity of depolarizing current pulse to trigger an action potential. After reperfusion, the spike threshold of both cell types was unchanged within the first 24 h but significantly increased at $\sim 30$ h ($P < 0.05$) and then returned to control value (Fig. 7, A and C), indicating a transient decrease in neuronal excitability after ischemia. The rheobase of CA3 neurons slightly fluctuated...
after reperfusion but was not significantly different from the preischemic values (Fig. 7B). The rheobase of granule cells, however, increased from 0.28 ± 0.16 nA before ischemia to 0.57 ± 0.36 nA ~31 h after reperfusion (P < 0.01) and then gradually returned to 0.34 ± 0.21 nA 7 days after reperfusion (Fig. 7D).

The changes in repetitive firing characteristics of CA3 and granule cells after ischemia were studied. Repetitive firing was induced by application of depolarizing current pulses (400 ms, 0.3–1.6 nA). In control animals, the spike-frequency adaptation of the spike trains in CA3 neurons was less obvious than that in granule cells, as indicated by the small difference in spike frequency between the first interspike interval (ISI) and the second ISI (Fig. 8). Within the first 24 h reperfusion, spike frequency of CA3 neurons dramatically reduced at all current pulse levels and then slowly returned to the preischemic value (Fig. 8, A and B). A similar transient decrease in spike frequency was also found in granule cells, but it occurred 24–36 h after reperfusion (Fig. 8, C and D).

The postischemic changes of fast afterhyperpolarization (fAHP) in CA3 and granule cells were also examined. The amplitude of fAHP was measured as deviation from the beginning of the upstroke of an action potential ≤5 ms after the peak of a single spike. The amplitude of fAHP dramatically increased from ~0.5 mV in control CA3 neurons to ~2 mV after reperfusion and remained at this level for 2 days (P < 0.05). The amplitude of fAHP returned to control values 7 days after reperfusion (Fig. 9, A and B). The amplitude of fAHP in control granule cells was much higher than that of CA3 neurons. After reperfusion, the amplitude of fAHP in granule cells showed a similar change as that of

![Image of current-voltage (I-V) relationship of CA3 neurons before and after ischemia. A–D: recordings at different intervals after reperfusion. Membrane potential deflections from a neuron recorded 4 h after reperfusion was smaller (B) when compared with that of control neuron (A), and the neurons at later time points (C and D) displayed the similar size of membrane potential deflection as compared with that of control neurons. Top panels: membrane potential deflections caused by the current pulses. Bottom panels: intracellular applied current pulses. Each trace is the average of 4 recordings. Scales in D apply to all traces. E: I-V curves of CA3 neurons before and at different periods after reperfusion. The values of each point are the means. A transient decrease in the slope of I-V was apparent at ~6 h after reperfusion, indicating an input resistance reduction during this period.](http://jn.physiology.org/doi/abs/10.1152/jn.1995.275.11.2865)

![Image of current-voltage (I-V) relationship of granule neurons before and after ischemia. A–D: top panels; membrane potential deflections caused by the current pulses. Bottom panels: intracellular applied current pulses. Each trace is the average of 4 recordings. In comparison with the control neuron (A), membrane potential deflections from a neuron recorded 34 h after reperfusion became significantly smaller than that of control one, indicating the decrease of input resistance (C). E: I-V curves in granule neurons before ischemia and at different periods after reperfusion. The values of each point are the means. The slope of I-V curve reduced at ~31 h after reperfusion and then returned to control levels, indicating a transient decrease of input resistance during this interval.](http://jn.physiology.org/doi/abs/10.1152/jn.1995.275.11.2865)
FIG. 7. Plots showing the changes in spike threshold and rheobase of CA3 neurons (A and B) and granule cells (C and D) at different periods after reperfusion. The value of each plot is the mean with the number of neurons in parentheses. The spike threshold of both CA3 and granule cells transiently increased ~30 h after reperfusion, indicating a transient decrease of neuronal excitability. No significant change in rheobase was observed in CA3 neurons. A significant increase in rheobase was found at ~31 h after reperfusion.

CA3 neurons, but no statistical significance was detected among these changes (Fig. 9, C and D).

DISCUSSION

This study characterized the temporal changes in synaptic transmission and membrane properties of CA3 and granule cells after a severe transient ischemia in an in vivo preparation. In contrast to CA1 neurons, both CA3 and granule cells exhibited a transient depression in synaptic transmission and neuronal excitability after ischemia. The different responses between ischemia-vulnerable and -resistant neurons after reperfusion may help to identify the neurophysiological mech-
FIG. 9. The changes in amplitude of fast afterhyperpolarization (fAHP) in CA3 and granule cells after ischemia. A and C: representative recordings showing fAHPs at different intervals after reperfusion. fAHP is the downward deflection of membrane potential immediately after action potentials. The scales in C apply to all traces in this figure. B: temporal profile of fAHPs in CA3 neurons after ischemia indicates a long-lasting significant increase in fAHP amplitude after reperfusion. The amplitude of fAHP returned to control levels 7 days after reperfusion. D: postischemic changes of fAHP in granule cells exhibited a similar pattern as that in CA3 neurons with no statistical significance. The numbers in parentheses are the number of neurons in each group.

anisms associated with neuronal damage after transient cerebral ischemia.

**Synaptic transmission in ischemia-vulnerable and -resistant neurons**

The changes of synaptic transmission in CA3 and granule cells after ischemia are significantly different from that of CA1 neurons. It has been shown that synaptic transmission in CA3 or granule cells was more resistant to hypoxia than CA1 neurons (Aitken and Schiff 1986; Balestrino et al. 1989; Cherubini et al. 1989; Kass and Lipton 1986). Previous studies with in vitro preparation reported a synaptic facilitation in CA1 area after hypoxia or ischemia (Crepel et al. 1993; Hori and Carpenter 1994; Urban et al. 1989). Recent studies with in vivo preparation have shown an enhancement of synaptic transmission in CA1 neurons after severe transient ischemia (Gao and Xu 1996; Gao et al. 1998a). A late-depolarizing postsynaptic potential (L-PSP) was induced from ~70% of CA1 neurons after reperfusion (Gao and Xu 1996). The amplitude and slope of initial EPSPs in these neurons significantly increased within first 12 h reperfusion (Gao et al. 1998a). In contrast, as shown in this study, the amplitude and slope of EPSPs in CA3 neurons significantly decreased within first 12 h reperfusion, indicating a transient suppression of synaptic transmission. The amplitude and slope of EPSPs in granule cells also decreased after ischemia but with less extent than CA3 neurons, suggesting that the granule cells are less sensitive to ischemic insult than CA3 neurons. It is not clear that to what extent the changes of synaptic transmission in hippocampal neurons after ischemia are due to the changes of membrane properties (i.e., whether changes in synaptic transmission are due to the changes of postsynaptic component). In this study, the depression of synaptic transmission in CA3 and granule cells is well correlated with the decrease of membrane input resistance and time constant after ischemia. However, in CA1 neurons, the neurons showing potentiation of synaptic transmission (L-PSP neurons) also exhibited a decrease of input resistance and time constant after ischemia (Gao et al. 1998a,b). Further studies are needed to clarify this issue.

The previous studies indicated that the ischemia-vulnerable and -resistant neurons displayed a completely different response to severe ischemia in terms of synaptic transmission: potentiation in CA1 neurons and suppression in CA3 and granule cells. Because most of the CA1 neurons die after such severe ischemia, it is conceivable that the enhancement of synaptic transmission plays a role in postischemic cell death. On the other hand, the depression of synaptic transmission may be associated with the mechanisms of survival after ischemia based on the observations: 1) the synaptic transmission was transiently suppressed in CA3 neurons that survive the severe ischemia as shown, and 2) the synaptic transmission was also transiently suppressed in CA1 and CA3 neurons after a mild sublethal ischemia (Howard et al. 1998; Xu and Pulsinelli 1994, 1996). If glutamate excitotoxicity plays a role in pathogenesis of posts ischemic cell death, it probably acts on enhancement of synaptic transmission rather than increase of neuronal firing rate. Further study is needed to elucidate how enhancement of synaptic transmission after ischemia induces neuronal damage.

**Membrane excitability in ischemia-vulnerable and -resistant neurons**

The pattern of excitability change in CA3 and granule cells after severe transient ischemia is different from that
of CA1 neurons. Previous study in brain slice from gerbil subjected to in vivo ischemia has shown that the excitability of CA1 neurons persistently decreased (Urban et al. 1989). By using in vivo preparation, a progressive suppression of excitability was also observed in most of CA1 neurons after severe transient ischemia (Gao et al. 1998b). In this study, however, only a transient increase in spike threshold and transient decrease in repetitive firing rate were observed in CA3 and granule cells after severe ischemic insult, indicating a transient depression of excitability in ischemia-resistant neurons. A slight difference between CA3 and granule cells is that the spike threshold of CA3 neurons increased during 24–36 h reperfusion, whereas the rheobase remained unchanged (Fig. 7, A and B). This is probably due to the increase of membrane input resistance and time constant of CA3 neurons during this period (Table 1). Interestingly, a transient depression of excitability was also observed in CA1 neurons after a mild ischemia that did not induce CA1 cell death (Xu and Pulsinelli 1996). These observations suggested that the persistent decrease in neuronal excitability may be related to posts ischemic cell death, whereas the transient decrease in neuronal excitability is probably the general reaction to an ischemic insult.

It has been shown that decreased excitability of CA1 neurons during anoxia is due to the increase of potassium conductance (Fujiwara et al. 1987; Hansen et al. 1982; Lebond and Krenjecv 1989). The decrease of neuronal excitability after severe ischemia may be also due to the increase of potassium conductance. In supporting this assumption, the amplitude of fAHP that is mediated by calcium-dependent potassium conductance progressively increased in CA1 neurons (Gao et al. 1998b) and transiently increased in CA3 neurons after severe ischemia (Fig. 9). The increase of fAHP amplitude after ischemia probably is also responsible for the decrease of spontaneous firing rate as well as the suppression of repetitive firing after reperfusion. The increase of potassium currents after reperfusion may account for the posts ischemic neuronal injury. A recent study demonstrated that the increase of potassium current induced apoptotic cell death in neurons (Yu et al. 1997). It is possible that persistent increase of potassium current after ischemia causes progressive depression of excitability and triggers the process of cell death. On the other hand, the depression of excitability may not be related to mechanisms of posts ischemic cell death. It may be a general response to ischemic stress. The surviving neurons recovering from the stress exhibit a transient change, whereas the degenerating neurons failed to recover from the insult and show a progressive depression of excitability.

This research was supported by American Heart Association AHA Grant 94008130 and National Institute of Neurological Disorders and Stroke Grant NS-33101 to Z. C. Xu. T. M. Gao is the recipient of postdoctoral fellowships from Neuroscience Center of Excellence at the University of Tennessee–Memphis and Grants NNSF 39570263 and NSFGP 950537 of People’s Republic of China.

Address for reprints: Z. C. Xu, Dept. of Anatomy, MSS07, Indiana University School of Medicine, 635 Barnhill Dr., Indianapolis, IN 46202.

Received 23 June 1998; accepted in final form 9 September 1998.

REFERENCES


Crepel, V., Ham mond, C., Chenestra, P., Dhabira, D., and Ben-Ari, Y. An LTP of NMDA receptor-mediated currents induced by anoxia. In press.


