Differential Changes of Potassium Currents in CA1 Pyramidal Neurons After Transient Forebrain Ischemia

XIAN XUAN CHI AND ZAO C. XU
Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, Indiana 46202
Received 5 June 2000; accepted in final form 16 August 2000

Chi, Xian Xuan and Zao C. Xu. Differential changes of potassium currents in CA1 pyramidal neurons after transient forebrain ischemia. J Neurophysiol 84: 2834–2843, 2000. CA1 pyramidal neurons are highly vulnerable to transient cerebral ischemia. In vivo studies have shown that the excitability of CA1 neurons progressively decreased following reperfusion. To reveal the mechanisms underlying the posts ischemic excitability change, total potassium current, transient potassium current, and delayed rectifier potassium current in CA1 neurons were studied in hippocampal slices prepared before ischemia and at different time points following reperfusion. Consistent with previous in vivo studies, the excitability of CA1 neurons decreased in brain slices prepared at 14 h following transient forebrain ischemia. The amplitude of total potassium current in CA1 neurons increased ~30% following reperfusion. The steady-state activation curve of total potassium current progressively shifted in the hyperpolarizing direction with a transient recovery at 18 h after ischemia. For transient potassium current, the amplitude was transiently increased ~30% at ~12 h after reperfusion and returned to control levels at later time points. The steady-state activation curve also shifted ~20 mV in the hyperpolarizing direction, and the time constant of removal of inactivation markedly increased at 12 h after reperfusion. For delayed rectifier potassium current, the amplitude significantly increased and the steady-state activation curve shifted in the hyperpolarizing direction at 36 h after reperfusion. No significant change in inactivation kinetics was observed in the above potassium currents following reperfusion. The present study demonstrates the differential changes of potassium currents in CA1 neurons after reperfusion. The increase of transient potassium current in the early phase of reperfusion may be responsible for the decrease of excitability, while the increase of delayed rectifier potassium current in the late phase of reperfusion may be associated with the posts ischemic cell death.

INTRODUCTION

CA1 pyramidal neurons in hippocampus start to degenerate 2–3 days after transient cerebral ischemia and 90% of them die in 1 wk (Kirino 1982; Pulsinelli et al. 1982). The mechanisms of this delayed cell death are under active investigation. It has been postulated that the neuronal hyperactivity due to an elevation of extracellular excitatory amino acids during ischemia leads to a massive increase in intracellular-free Ca2+, which may trigger the process of neuronal degeneration (Choi and Rothman 1990; Rothman and Olney 1986). To test the excitotoxic hypothesis, many investigators examine the neuronal activity during hypoxia/ischemia and after reperfusion. It has been shown that the neuronal activity was reduced and the excitability was depressed during hypoxia in vitro (Boissard and Gribkoff 1993; Fujiwara et al. 1987; Hansen et al. 1982; Leblond and Krenjiev 1989) and during ischemia in vivo (Xu and Pulsinelli 1994, 1996). Because the elevated extracellular concentration of glutamate and aspartate returns to control levels 30 min after reperfusion (Benveniste et al. 1984; Mitani et al. 1992) and the CA1 neurons begin to die in 2–3 days, the changes of neuronal activity after reperfusion may be more important in the process of cell death than those during ischemia. Due to the technical limitations, it is difficult to study the electrophysiological changes after reoxygenation in an in vitro preparation. Using brain slices prepared at different intervals following ischemia in vivo, Urban et al. (1989) have shown that the population spike was unchanged or reduced 5–10 h after reperfusion and was markedly depressed by 24 h reperfusion, suggesting the depression of neuronal excitability following reperfusion. Using extracellular recording techniques in vivo, early studies have shown that the neuronal firing rate increases in CA1 region following reperfusion (Chang et al. 1989; Suzuki et al. 1983). More studies, on the other hand, report a decrease of neuronal firing rate in CA1 region after reperfusion (Buzsák et al. 1989; Furukawa et al. 1990; Mitani et al. 1990). Using intracellular recording and staining techniques in vivo, recent studies have clearly demonstrated that the spontaneous neuronal activity and excitability of CA1 pyramidal neurons significantly decreased ≤48 h following transient forebrain ischemia (Gao et al. 1998, 1999).

Potassium currents are important for the regulation of neuronal excitability and the maintenance of baseline membrane potential (Brown et al. 1990; Hille 1992; Storm 1990). The regulation of potassium channel activity is believed to have a major impact on the overall neuronal response and adaptation to O2 deprivation (Haddad and Jiang 1993). During hypoxia in vitro, the extracellular potassium markedly increases (Haddad and Donnelly 1990). Activation of potassium channels induces hyperpolarization, decreases membrane excitability, and reduces O2 consumption (Belousov et al. 1995; Croning et al. 1995; Fujimura et al. 1997; Haddad and Jiang 1993; Yamamoto et al. 1997). The alteration of potassium conductance during hypoxia has been shown to be responsible for the prevailing hyperpolarization, possible through several different types of conductance including Ca2+-sensitive potassium cur-
In the present study, the hippocampal slices were prepared at different intervals after ischemia in vivo. The membrane properties of CA1 neurons after reperfusion were studied to confirm the posts ischemic changes in our in vitro preparation are comparable to those observed in in vivo preparation. Then the temporal profiles of amplitude and kinetics of different voltage-dependent potassium currents were examined ≤36 h following reperfusion to reveal their roles in excitability changes in CA1 pyramidal neurons following transient forebrain ischemia.

METHODS

Transient forebrain ischemia

Male adult Wistar rats (150–200 g) were used in the present study. The National Institutes of Health guides for the care and use of laboratory animals were strictly followed. Transient forebrain ischemia was induced using the four-vessel occlusion method (Pulsinelli and Brierley 1979) with modifications (Xu et al. 1999). Briefly, the animals were fasted overnight and anesthetized with 1–2% halothane mixed with 33% O₂ and 66% N₂. The vertebral arteries were electrocauterized. The common carotid arteries were isolated after which an occluding device was placed loosely around each carotid artery to allow subsequent occlusion of these vessels. The animal was then placed on a stereotaxic frame and a temperature probe (0.025-in diam) was placed beneath the skull in the extradural space, after which brain temperature was maintained at 37°C with a heating lamp through a temperature control unit (BAT-10, Physitemp). Severe forebrain ischemia was produced by occluding both common carotid arteries to induce ischemic depolarization for ~14 min. Animals were returned to the cage after recovering from ischemia and allowed free access to water and food. The animals were then re-anesthetized and prepared for brain slices at different time points after reperfusion.

Electrophysiology

Brain slices were prepared from animals before ischemia and at 6–8, 12–14, 18–20, and 36–38 h following reperfusion as described in previous publications (Chi and Xu 2000). The animals were anesthetized with ketamine-HCl (13 mg/kg) and decapitated. The brains were quickly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF), which was composed of the following (in mM): 130 NaCl, 3 KCl, 2 CaCl₂, 2 MgCl₂, 1.25 NaH₂PO₄, 26 NaHCO₃, and 10 glucose. Hippocampal slices of 300 μm thickness were cut using a vibroslice (Campden 752 M), and were incubated in ACSF for $\sim$15 min at room temperature before transferring to recording chamber. The slice was submerged beneath the fluid surface and superfused continuously with oxygenated ACSF. The flow rate was adjusted to 2–3 ml/min. Recordings were carried out at room temperature (~24°C). Tetrodotoxin (1 μM) and cadmium chloride (0.3 mM) were added into the solution to block Na⁺ and Ca²⁺ channels.

For intracellular recording, the recording electrodes were pulled from glass capillaries with filaments to a tip resistance of 50–80 MΩ.

TABLE 1. Membrane properties of CA1 neurons before and after ischemia

<table>
<thead>
<tr>
<th></th>
<th>RMP, mV</th>
<th>SpkH, mV</th>
<th>SpkD, ms</th>
<th>SpkT, mV</th>
<th>Rheobase, nA</th>
<th>R_{in}, MΩ</th>
<th>T_{cons}, ms</th>
<th>fAHP, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>−68 ± 1.62 (12)</td>
<td>88 ± 2.23 (13)</td>
<td>1.11 ± 0.06 (13)</td>
<td>−54 ± 0.86 (13)</td>
<td>0.3 ± 0.03 (18)</td>
<td>28 ± 1.35 (20)</td>
<td>9.81 ± 0.66 (15)</td>
<td>4.7 ± 0.37 (20)</td>
</tr>
<tr>
<td>Ischemia</td>
<td>−62 ± 1.93* (17)</td>
<td>83 ± 1.62 (22)</td>
<td>0.95 ± 0.05* (22)</td>
<td>−51 ± 1.15* (20)</td>
<td>0.7 ± 0.09* (22)</td>
<td>23 ± 1.25* (22)</td>
<td>7.91 ± 0.62* (15)</td>
<td>9.4 ± 0.48* (26)</td>
</tr>
</tbody>
</table>

Values are means ± SE, with number of neurons in parentheses. Rheobase is determined as the minimal intensity of depolarizing current pulse to trigger action potentials. Input resistance ($R_{in}$) is derived from the linear portion of the current-voltage curve (0–0.5 nA). Time constant ($T_{cons}$) is derived from transients of hyperpolarizing pulse (~0.3 nA, 200 ms). RMP, resting membrane potential; SpkH, spike height, measured from the resting membrane potential; SpkD, spike width measured at half of the peak amplitude of the action potential; SpkT, spike threshold measured at the beginning of the upstroke of the first action potential; fAHP, fast afterhyperpolarization. * P < 0.05.

FIG. 1. An example of intracellularly stained CA1 pyramidal neuron in hippocampus at 36 h after reperfusion. The beaded dendrite (↓) is evident in small apical dendrites. The soma and basal dendrites are not in focus because the focus was adjusted at the apical dendrites.
when filled with a solution of 3% neurobiotin (Vector) in 2 M potassium acetate. The microelectrode was advanced slowly into the CA1 pyramidal cell layer. After impalement, the neurons with a stable membrane potential of $-60 \text{ mV}$ or greater were selected for further study.

For whole cell recording, patch electrodes were prepared from borosilicate glass (Warner Instrument) to produce tip openings of 1–2 $\mu \text{m}$ (3–5 $\text{M}\Omega$). Electrodes were filled with an intracellular solution containing (in mM) 145 KCl, 1 MgCl$_2$, 10 EGTA, 0.2 CaCl$_2$, and 10 HEPES buffer (Sigma) and 3% neurobiotin (Vector). CA1 neurons were visualized with an infrared-DIC microscope (Olympus BX 50 WL) and a CCD camera. Positive pressure was applied to recording pipette as it was lowered into the medium and approached the cell membrane. Constant negative pressure was applied to form the seal ($>1 \text{ G}\Omega$) when the recording pipette attached the membrane. A sharp pulse of negative pressure was applied to open the cell membrane for whole cell recording.

Voltage-clamp recording was performed with an Axopatch 200 B amplifier (Axon Instruments). Pipette tip junction potentials were continuously monitored and compensated as necessary before breakage of the membrane. No leak current subtraction was performed because we found that the leak current was much smaller than the currents activated by depolarization. Cells were voltage-clamped and held near resting membrane potential (about $-60 \text{ mV}$). In brain slice preparation, CA1 pyramidal neurons are not fully space-clamped due to the extensive dendritic trees. However, it has been shown that the lack of voltage control in the dendrites did not dramatically alter the kinetics of potassium current (Surmeier et al. 1994). Furthermore the poor space-clamp will not invalidate the outcome of the present study because the comparison was based on the data collected under the same preparation before and after ischemia. Signals were filtered at 5

![FIG. 2. Current-voltage (I-V) relationship of CA1 pyramidal neurons before ischemia and after reperfusion. A: representative recordings of I-V relationship of CA1 neurons. The traces are average of 4 recordings. B: plots of I-V curve from control and ischemic neurons. The slope of the I-V curve of ischemia neuron is smaller than that of control indicating the reduction of input resistance.](http://jn.physiology.org/)

![FIG. 3. Comparison of total potassium current before and after ischemia. A: examples of total potassium currents evoked from CA1 pyramidal neurons before and after reperfusion. The voltage protocol is shown at the bottom. B: peak amplitude of total potassium current in CA1 neurons before and after reperfusion. The amplitude of total potassium current after reperfusion is significantly higher than that of control neurons. C: comparison of steady-state activation of total potassium current before and at different time points after reperfusion. The steady-state activation curve of total potassium current progressively shifts in the hyperpolarizing direction except a transient recovery at 18 h after reperfusion. D: steady-state inactivation of total potassium current before and after reperfusion. No significant change in inactivation curve is observed after reperfusion.](http://jn.physiology.org/)
2 groups) or ANOVA (for more than 2 groups) was used for statistical analysis. The transient potassium current is isolated by subtraction of the current evoked after TEA application from the control one.

Additional application of 10 mM TEA (0.1 M potassium phosphate-buffered saline (pH 7.4) with 0.5% Triton X-100 for 24 h at room temperature. After detection of potassium activity with 3,3'-diaminobenzidine, slices were examined in potassium phosphate buffered saline. Slices containing labeled neurons were mounted on gelatin-coated slides and processed for light microscopy.

**Data analysis**

To establish steady-state activation or inactivation curves, the peak current (I) was measured at each potential and the corresponding conductance (G) was calculated with the use of the following equation:

$$G = I / (V_m - V_h)$$

where $V_m$ was the membrane command potential and $V_h$ was the reversal potential ($V_h = -98$ mV). The measured peak amplitudes and the calculated peak conductance were then normalized with respect to the maximum values and plotted as a function of the membrane potential during the test pulse. The resulting activation and inactivation curves were fitted by the Boltzmann equation:

$$G / G_{\text{max}} = \left( 1 + \exp \left[ \left( V_{1/2} - V / c \right) / k \right] \right)^{-1}$$

where $G_{\text{max}}$ is the maximal conductance obtained at $+70$ mV, $V_{1/2}$ is the membrane voltage at which the current amplitude is half-maximum and $V_c$ is the slope factor at $V_{1/2}$.

The values were presented as means ± SE. The Student’s t-test (for 2 groups) or ANOVA (for more than 2 groups) was used for statistical analysis (Statview, Abacus Concepts).

**Results**

Experiments were performed on control rats ($n = 25$) and rats subjected to forebrain ischemia with ischemic depolarization of 13.8 ± 0.9 min ($n = 45$). Such degree and duration of ischemia consistently produced 90% cell death in the CA1 region 1 wk after reperfusion (Xu et al. 1999). A total of 81 neurons were analyzed in the present study of which 60 were successfully stained and identified as CA1 pyramidal cells. No overt degeneration signs, such as swelling or shrinkage of cell body and dendritic fragmentation, were observed in these neurons. However, small beaded dendrites were observed in apical dendrites of some neurons recorded at 36 h following reperfusion, suggesting the beginning of degeneration at this time (Fig. 1). Two glial cells and five interneurons were morphologically identified and were excluded from the analysis.

Whole cell recording of CA1 neurons became very difficult at 2 days after reperfusion, especially in the medial portion of the CA1 zone. Hematoxylin-eosin staining was performed in four animals at 48 h after reperfusion, signs of neuronal death were evident in the medial portion of CA1 region.

**Membrane properties of CA1 neurons in brain slice following reperfusion**

The membrane properties of CA1 pyramidal neurons were compared before ischemia and at 14–16 h following reperfusion. As shown in Table 1, the resting membrane potential of CA1 neurons depolarized from $-68 ± 1.62$ mV of control value to $-62 ± 1.93$ mV after reperfusion ($P < 0.05$). The spike width was decreased from 1.11 ± 0.06 to 0.95 ± 0.05 ms ($P < 0.05$). No significant difference in spike height was observed after reperfusion.

The changes in neuronal excitability were evaluated by comparing the spike threshold and rheobase of CA1 neurons before ischemia and after reperfusion. The spike threshold ($-54 ± 0.68$ vs. $-51 ± 1.15$ mV, $P < 0.05$), and rheobase ($0.3 ± 0.03$ vs. $0.7 ± 0.09$ nA, $P < 0.05$) of CA1 neurons increased after reperfusion, indicating the decrease of excitability.

To compare the current-voltage relationship of CA1 neurons before and after reperfusion, constant-current pulses (200 ms, −1.0 to +0.5 nA, 0.1-nA increment) were delivered. The voltage values were measured from the averages of four recordings at the steady state of the transients (average between 160 and 180 ms from the beginning of the pulse). The slope of I-V curve in CA1 neurons significantly decreased after reperfusion indicating the decrease of input resistant (Fig. 2).

The postischemic change of fast afterhyperpolarization (fAHP) in CA1 neurons was also examined. The amplitude of fAHP was measured from the beginning of the upstroke of an action potential to the most hyperpolarizing point within 5 ms after the peak of the action potential. The amplitude of fAHP was reduced significantly after ischemia (90% determined in 10 min) followed by a gradual recovery (75% after 3 h).
Increased from 4.7 ± 0.37 mV of control level to 9.4 ± 0.48 mV at 14–16 h after reperfusion (P < 0.05).

**Total potassium current in CA1 neurons after reperfusion**

To activate outward currents, membrane potential was held at −60 mV and stepped voltage command pulses (−80 − +70 mV, 10 mV/step, 400 ms) were applied following a conditioning voltage step of 300 ms at −120 mV. Outward currents became apparent at approximately −55 mV, and their amplitude increased at more depolarizing potentials (Fig. 3A). The amplitude of total potassium current was measured at 20 ms after the onset of command pulses. The peak amplitude of total potassium current significantly increased from 2.31 ± 0.24 nA of control to 3.08 ± 0.22 nA at 12 h after reperfusion (measured at +70 mV, P < 0.05) and slightly reduced at later time points (Fig. 3B).

The activation curve was obtained by fitting the data points with a Boltzmann equation. Figure 3C shows that the steady-state activation curve of total potassium current progressively shifted in hyperpolarizing direction with a transient recovery at 18 h after ischemia. The slope of the activation curves did not change after reperfusion (Table 2).

Steady-state inactivation of total potassium current was studied by holding the cell for 2 s at potentials between −150 and −10 mV prior to delivering a testing pulse to +30 mV. Normalized peak current amplitude as a function of conditioning potential was fitted by a Boltzmann equation. No significant difference in \( V_{1/2} \) and \( V_c \) was found in CA1 neurons after reperfusion (Fig. 3D, Table 2).

**FIG. 5.** A: example of transient potassium currents evoked from CA1 neuron before ischemia and after reperfusion. Transient potassium current is isolated by subtraction of the current evoked by voltage protocol P1 from that evoked by protocol P2. B: peak amplitude of transient potassium currents in CA1 neurons before and after reperfusion. The amplitude of transient potassium current in neurons at 6–8 and 12–14 h after reperfusion is significantly higher than that of control ones. C: comparison of the current density of transient potassium current at different time points after reperfusion. The current density of transient potassium current at 12–14 h after reperfusion is significantly higher than that of control value.

**FIG. 6.** A: comparison of steady-state activation of transient potassium current before and after reperfusion. The steady-state activation curve shifts in the hyperpolarizing direction within 12 h after reperfusion and slightly returns toward the control value. B: steady-state inactivation of transient potassium current before and after reperfusion. No significant difference is found in inactivation curves after reperfusion.
Pharmacological intervention allows us to dissect the different components of potassium current and to investigate their gating properties respectively. The total potassium current was reduced ~60% (1.52 ± 0.20 vs. 0.66 ± 0.08 nA, n = 12, measure at +30 mV, P < 0.01) after bath application of tetraethylammonium (TEA, 30 mM, Fig. 4A). A slow inactivating component was evident after subtracting the current following TEA application from the control one (Fig. 4B). This component showed little inactivation and resembles delayed rectifier current (Storm 1990) and indicated as $I_{Kd}$. After TEA application, a transient component was also identified in the total outward current. Bath application of 4-aminopyradine (4-AP, 10 mM) completely blocked this component (Fig. 4A). The transient component was isolated by subtracting the current following TEA and 4-AP application from the control one (Fig. 4C). This transient component reached its peak within 20 ms following the onset of membrane depolarization and rapidly inactivated despite the continued membrane depolarization. The inactivation profile was best fitted by a monoexponential function. The time constant for the inactivation was 10.03 ± 1.49 ms (n = 12). This current resembles a transient potassium current and refereed as $I_A$ (Storm 1990). No attempt was made to distinguish the slow and fast components of $I_A$ in the present study.

**Transient potassium current in CA1 neurons after reperfusion**

$I_A$ was isolated by subtracting slow component from the total potassium current (Klee et al. 1997; Numann et al. 1987). Membrane potential was held at ~60 mV. Depolarizing potential steps were preceded by 300 ms hyperpolarizing pulse at −120 mV (Fig. 5A, voltage protocol $P_1$) to evoke outward currents including $I_A$. $I_A$ was inactivated by a 50-ms prepulse at +10 mV (Fig. 5A, voltage protocol $P_2$). $I_A$ was obtained by subtracting current evoked from $P_2$ from that evoked from $P_1$. The amplitude of $I_A$ was measured at the peak of the current (at ~20 ms after the onset of testing pulses). The threshold for activating $I_A$ was around −55 mV. The amplitude of $I_A$ progressively increased from 0.42 ± 0.06 nA of control to 0.70 ± 0.09 nA (measured at +30 mV, P < 0.05) at 12 h after reperfusion. The amplitude returned to control level 18–36 h after reperfusion (Fig. 5B).

In whole cell patch-clamp study, the current amplitude may vary due to the variation of cell body volume. In an attempt to eliminate the changes of current amplitude resulting from the changes of cell volume, which may occur after ischemic insult, the current density of $I_A$ was compared before ischemia and after reperfusion. The membrane capacitance, which indirectly represents the cell volume, was measured. The current amplitude of $I_A$ measured at command pulses of +30 mV was divided by the membrane capacitance of each individual neuron yielding measurement of current density (expressed as pA/pF). The average current density was 32.9 ± 4.2 pA/pF for control and 54.6 ± 7.7 pA/pF for neurons at 12–14 h after reperfusion (n = 5, P < 0.05, Fig. 5C).

Activation curves of $I_A$ shifted in hyperpolarizing direction within 12 h after reperfusion and returned to the control level at 18–36 h reperfusion. The $V_{1/2}$ shifted from −6.31 ± 1.48 mV of control value to −27.03 ± 1.24 mV at 12 h after reperfusion (P < 0.05, Fig. 6A). Mean $V_c$ values were similar across different time points following reperfusion (Table 3). The steady-state inactivation properties of $I_A$ were determined by measuring current availability following 400-ms prepulses steps to voltage between −120 and 0 mV with a test pulse to +30 mV. No significant change in $V_{1/2}$ and $V_c$ of inactivation

### TABLE 3. Kinetics of transient potassium current before and after ischemia

<table>
<thead>
<tr>
<th>Condition</th>
<th>$V_{1/2}$ (Act.), mV</th>
<th>$V_c$ (Act.), mV</th>
<th>$V_{1/2}$ (Inact.), mV</th>
<th>$V_c$ (Inact.), mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>−6.31 ± 1.48</td>
<td>14.63 ± 1.20</td>
<td>−59.58 ± 1.02</td>
<td>13.23 ± 0.92</td>
</tr>
<tr>
<td>6–8 h</td>
<td>−29.67 ± 1.53*</td>
<td>14.67 ± 1.31</td>
<td>−65.50 ± 0.90</td>
<td>15.49 ± 0.81</td>
</tr>
<tr>
<td>12–14 h</td>
<td>−27.03 ± 1.24*</td>
<td>12.34 ± 1.06</td>
<td>−63.32 ± 0.76</td>
<td>14.75 ± 0.69</td>
</tr>
<tr>
<td>18–20 h</td>
<td>−17.55 ± 1.54</td>
<td>15.08 ± 1.27</td>
<td>−61.88 ± 0.67</td>
<td>11.10 ± 0.59</td>
</tr>
<tr>
<td>36 h</td>
<td>−17.49 ± 0.85</td>
<td>12.73 ± 0.71</td>
<td>−72.82 ± 0.65</td>
<td>14.03 ± 0.59</td>
</tr>
</tbody>
</table>

$V_{1/2}$, the potential of half-maximal activation or inactivation; $V_c$, proportional to the slope at $V_{1/2}$; * P < 0.05.
curve was detected in CA1 neurons after reperfusion (Table 3, Fig. 6B).

To determine the removal of inactivation of $I_A$, the cells were held at $-20$ mV to inactivate $I_A$, and the membrane potential was then stepped to $-120$ mV for periods between 0 and 200 ms to remove inactivation of $I_A$ prior to a test pulse of $+70$ mV. The *inset* in Fig. 7A shows a representative recording of the time-dependent recovery from inactivation of $I_A$. Normalized peak $I_A$ was plotted against prepulse duration to reveal the time course of recovery from inactivation. The curve was fitted by a single exponential function with the time constant of $42.39 \pm 6.67$ ms for control neurons and increased to $96.76 \pm 16.52$ ms for neurons at 12 h after reperfusion ($P < 0.05$). The time constant returned to control level at 36 h after reperfusion (Fig. 7B).

**Delayed rectifier potassium current in CA1 neurons after reperfusion**

In the present study, the $I_{Kd}$ was isolated by inactivating the fast transient components of the total potassium current (Klee et al. 1997; Numann et al. 1987). With holding potential at $-60$ mV, $I_{Kd}$ were elicited by a protocol where hyperpolarization or depolarization were separated by a 50-ms prepulse at $+10$ mV, which inactivated the transient potassium current (Fig. 8A). The amplitude of the $I_{Kd}$ gradually increased and reached the peak at 36 h after reperfusion ($P < 0.05$, Fig. 8B). No significant difference in membrane capacitance was found in these neurons (12.85 $\pm$ 0.33 pF for control and 12.68 $\pm$ 0.18 pF at 36 h after reperfusion), suggesting that the increase of $I_{Kd}$ current after reperfusion was not due to the increase of cell volume. The activation curve shifted $12$ mV in the hyperpolarizing direction ($V_{1/2}: -4.95 \pm 1.92$ mV for control and $-16.36 \pm 1.64$ mV at 36 h after reperfusion; $P < 0.05$, Fig. 8C). No significant change in $V_{1/2}$ and $V_c$ of inactivation curves was found after reperfusion (Fig. 8D, Table 4).

**DISCUSSION**

The present study has shown that the excitability of CA1 neurons decreases in brain slices 14–16 h after reperfusion, which is coincide with results from previous studies using in

**Table 4. Kinetics of delayed rectifier potassium current before and after ischemia**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>6–8 h</th>
<th>12–14 h</th>
<th>18–20 h</th>
<th>36 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{1/2}$ (Act), mV</td>
<td>$-4.95 \pm 1.92$</td>
<td>$-8.10 \pm 2.26$</td>
<td>$-1.80 \pm 2.63$</td>
<td>$-3.12 \pm 2.03$</td>
<td>$-16.36 \pm 1.64^*$</td>
</tr>
<tr>
<td>$V_c$ (Act), mV</td>
<td>$21.33 \pm 1.44$</td>
<td>$21.51 \pm 1.73$</td>
<td>$24.16 \pm 1.84$</td>
<td>$22.35 \pm 1.48$</td>
<td>$19.08 \pm 1.34$</td>
</tr>
<tr>
<td>$V_{1/2}$ (Inact), mV</td>
<td>$-63.44 \pm 1.36$</td>
<td>$-66.67 \pm 1.20$</td>
<td>$-65.29 \pm 1.46$</td>
<td>$-65.09 \pm 2.23$</td>
<td>$-71.08 \pm 1.20$</td>
</tr>
<tr>
<td>$V_c$ (Inact), mV</td>
<td>$22.52 \pm 1.26$</td>
<td>$21.15 \pm 1.10$</td>
<td>$21.49 \pm 1.35$</td>
<td>$23.75 \pm 2.10$</td>
<td>$22.98 \pm 1.11$</td>
</tr>
</tbody>
</table>

$V_{1/2}$, the potential of half-maximal activation or inactivation; $V_c$, proportional to the slope at $V_{1/2}$; * $P < 0.05$. 
vivo preparation. Our results also demonstrate a differential change of potassium currents to ischemia insult. Transient potassium current (I_A) was transiently enhanced at ~12 h reperfusion whereas the delayed rectifier potassium current (I_Kd) was enhanced at ~36 h reperfusion. The increase of I_A at the early phase of reperfusion may be responsible for the decrease of excitability during this period, and the increase of I_Kd at the late phase of reperfusion may be associated with posts ischemic cell death in hippocampus.

Alteration of transient potassium current

The enhancement of I_A in CA1 neurons following reperfusion as demonstrated in the present study is different from previous studies on I_A during hypoxia. Cummins et al. (1991) reported that no consistent change of voltage-dependent potassium current was observed in isolated CA1 neurons during hypoxia, and others have shown the decrease of I_A in CA1 neurons during hypoxia (Gebhardt and Heinemann 1999; Hyllienmark and Brismar 1996). The conflicting results between previous reports and our observation may reflect the difference between changes during hypoxia and after reperfusion. It may also stem from the difference in preparation [dissociated or cultured neurons (Cummins et al. 1991; Gebhardt and Heinemann 1999) vs. brain slice] and the age of the animal [early postnatal (Hyllienmark and Brismar 1996) vs. adult]. Our results, on the other hand, are in line with many studies showing the increase of potassium currents during hypoxia (Boissard and Gibrkoff 1993; Fujiwara et al. 1987; Hansen et al. 1982; Leblond and Krenjovic 1989). It is conceivable that the decrease of excitability in CA1 neurons during hypoxia and after reperfusion may be governed by the same mechanism (i.e., increase of potassium conductance).

One possible mechanism underlying the enhancement of potassium currents is that the number of K^+ channels in CA1 neurons increases after reperfusion. It has been shown that the Na^+ channel mRNA and protein levels increase in immature brain but decrease in adult animals, suggesting that the number of ion channel could be altered by hypoxia or ischemia (Xia and Haddad 1999). However, considering the fact that protein synthesis in vulnerable neurons was inhibited after ischemia, especially during early reperfusion (Lipton 1999; Schmidt-Kastner and Freund 1990), it is unlikely the enhancement of I_A at the early phase of reperfusion is due to the increase number of potassium channels. The other possibility is that the channel properties, such as the open probability and opening time, have been altered after ischemia. It has been shown that membrane proteins including ion channels are responsive to their redox state (Bertl and Slayman 1990). Changes in the redox state of amino acid residues in channel proteins may lead to a conformational change and hence alters the channel activity (Ruppersberg et al. 1991). Ischemia could induce the change of redox state and subsequently alter the channel activity (Gozlan et al. 1994). It is possible that the channel open probability and opening time increase after ischemia and result in the enhancement of I_A current after reperfusion.

The transient potassium current is important to determine the spike threshold because it is activated near the resting membrane potential range and affects the latency of first spike (Brown et al. 1990; Hille 1992; Segal et al. 1984; Storm 1990). Increase of I_A will affect the spike threshold and therefore decrease the excitability of the neuron. I_A also partially contributes to the repolarization of the action potential (Ficker and Heinemann 1992). The increase of I_A will shorten the duration of action potential, reduce sodium and calcium influx, hence decrease the excitability of the neuron. In the present study, the duration of action potential significantly reduced after reperfusion; this is coincide with the increase of I_A conductance. The increase of transient potassium current therefore may be a major contributor to the decrease of excitability in CA1 neurons during the early period of reperfusion.

The increase of potassium conductance during early reperfusion may be an attempt to protect the neurons from ischemic insult. Some studies have showed that the activation of ATP dependent K^+ channel (K_ATP) during anoxia may be of importance in the response and adaptation of neurons (Ben Ari 1990; Jiang and Haddad 1991; Jiang et al. 1992). Activation of K_ATP channels on postsynaptic membrane of hypoglossal neurons tends to hyperpolarize or limit the depolarization of these neurons (Jiang and Haddad 1991). Hyperpolarization would reduce energy consumption and prevent activation of several cation channels, reduce energy expenditure, and have a protective effect when neurons are exposed to O_2 deprivation (Haddad and Jiang 1993). It has been demonstrated that application of K_ATP channel activator can protect neurons from focal or global ischemia (Heurteaux et al. 1993; Takaba et al. 1997). Because I_A is not a major contributor to resting membrane potential, its protective effect on neurons following reperfusion probably is by saving energy through increase spike threshold and decrease excitability rather than hyperpolarizing the resting membrane potential.

Alteration of delayed rectifier potassium current

The forebrain ischemia induced in the present study consistently produces cell death in 90% of CA1 pyramidal neurons 1 wk after reperfusion (Xu et al. 1999). In recent years, accumulating evidence has indicated that apoptosis, in addition to necrosis, is involved in neuronal damage after ischemia (MacManua and Linnik 1997; Schreider and Baudry 1995; Zeng and Xu 2000). It has been shown that the enhancement of outward potassium current is associated with neuronal apoptosis induced in different experimental settings including those mimicking ischemia conditions (Yu et al. 1997, 1998, 1999b). Using potassium channel blocker or raising extracellular potassium concentration reduces such apoptotic cell death. These studies suggest that potassium efflux is an important mediator of neuronal apoptosis. Further studies have indicated that the enhancement of potassium efflux, perhaps in particular via the delayed rectifier potassium current (I_Kd), participates apoptotic cell death (Yu et al. 1998, 1999a). However, a study using single-channel recording has shown that the amplitude, open probability, and mean opening time of I_Kd channel decreased in cerebellar granule cells 12 h after hydrogen peroxide application, which induced apoptosis (Chi et al. 1998). The preceding studies indicate that I_Kd is involved in apoptosis, but its role is complex. Its activity may increase or decrease depending on many factors such as the difference in insult and the time course of apoptosis.

Given the property of very slow inactivation, I_Kd contributes to extracellular K^+ accumulation and the reduction of intracellular potassium during ischem/hypoxia (Yu et al. 1997).
Potassium efflux leading to a reduction of intracellular potassium has been suggested to mediate several forms of apoptosis (Bortner et al. 1997; Hughes et al. 1997; Yu et al. 1997). Intracellular potassium at the normal levels inhibits caspase-3-like protease activation and apoptotic DNA fragmentation while reduction of intracellular potassium activates caspases and nucleases that play a central role in apoptotic cell death (Alnemri et al. 1996; Hughes et al. 1997; Yu et al. 1998; Yuan et al. 1993). Therefore the increase of delayed rectifier potassium current at 36 h reperfusion may be involved in apoptotic cell death after reperfusion.

We thank Dr. G. Nicol for technical help and constructive comments during the study.

This research was supported by National Institute of Neurological Disorders and Stroke Grant NS-38053 and American Heart Association Grants 0070048 and Stroke Grant NS-38053 and American Heart Association Grants 0070048 and 9920468 to X. X. Chi.

REFERENCES


