Morphological Heterogeneity of CA1 Pyramidal Neurons in Response to Ischemia

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We have found, based on the electrophysiological properties, two subtypes of CA1 pyramidal neurons in the CA1 region of the normal hippocampus, late postsynaptic potential (L-PSP) neurons and non-L-PSP neurons. In addition, our previous study has shown that the electrophysiological properties of these two subtypes of pyramidal neurons were differentially modified after ischemia. In the present study, we hypothesized that ischemia might also induce different morphological alterations in these two subtypes of neuron. To test the hypothesis, we compared the changes in the dendritic arborization and soma volume of these two subtypes of neurons in rats subjected to transient global ischemia. We found a significant decrease in the basal dendritic length of L-PSP neurons at 12 hr after reperfusion, resulting mainly from a significant decrease in the dendrite terminal length. The apical dendritic length of L-PSP neurons markedly increased at 24 hr after ischemia, resulting mainly from an increase in the number of branching arbors in the middle part of the apical dendritic trees. The soma size of L-PSP neurons was significantly reduced at 12 hr, but they became slightly larger at 24 hr and 48 hr after reperfusion. In contrast to L-PSP neurons, non-L-PSP neurons showed slight modifications in the dendritic arborization but had persistent swelling of their soma after ischemia. These results indicate that pathological changes in these two subtypes of neurons are different after ischemia.

Key words: hippocampus; stroke; reconstruction; dendrite; soma

Neuronal dendrites are major sites of synaptic inputs processing and integration. Dendritic branching patterns determine a neuron’s morphological complexity and impact signal transmission (Vetter et al., 2001). Different types of neurons have different dendritic patterns. For example, Purkinje cells in the cerebellum have an asymmetric arborization of dendrites located only in the molecular layer, whereas pyramidal neurons in the cortex and the hippocampus divide their dendrites into the basal and apical trees, which extend into different layers (Berry and Bradley, 1976; Larkman, 1991; Bannister and Larkman, 1995). Such differences in the dendritic branching patterns reflect the specific spatial connection and electrophysiological properties of a neuron (Mainen et al., 1996; Henze et al., 2000; Vetter et al., 2001).

Dendritic morphology of CA1 pyramidal neurons has been studied in normal rats and in monkeys by many investigators (Larkman, 1991; Ishizuka et al., 1995; Pyapali et al., 1998; Altemus et al., 2005). Although the quantitative data in these studies are relatively comparable, there are still considerable differences across the studies. The main causes for these differences likely are the methodological issue and the species issue. However, even in the same experiment, there is still wide variation in the dendritic trees of the pyramidal neurons in the visual cortex and CA1 region (Larkman, 1991; Bannister and Larkman, 1995), which suggests that there might be different subtypes of pyramidal neurons in these areas. However, little was known about the possibility of different subtypes of pyramidal neurons in the intact CA1 region until recently (Fan et al., 2005). In the Fan et al. study, about 70% of CA1 pyramidal neurons exhibited a late postsynaptic potential (L-PSP) in response to the stimulation on the Schaffer collaterals or the contralateral commissural path, whereas other CA1 pyramidal neurons did not elicit this response.

Neurons in the hippocampus and striatum are highly vulnerable to ischemia, but the latency of neuronal death in these areas after ischemia is different. All small to medium-sized spiny neurons in the striatum die rapidly within 1 day after transient global ischemia, but most CA1 pyramidal neurons die 3–4 days after ischemia (Pulsinelli et al., 1982; Kirino and Sano, 1984; Crain...
et al., 1988). These phenomena indicate heterogeneity of the response to ischemia in different types of neurons. Recently, we have found that ischemia induces dendritic plasticity of CA1 pyramidal neurons but that the morphological alterations vary considerably from cell to cell (Ruan et al., 2006). In addition, the previous study has shown that the changes in the electrophysiological properties in L-PSP and non-L-PSP neurons in the CA1 area are also different after ischemia (Gao and Xu, 1996). Therefore, we hypothesize that the morphological alteration induced by ischemia is different between L-PSP and non-L-PSP neurons. To test the hypothesis, in the present study we compared the changes in dendritic arborization as well as the soma volume within 2 days after ischemia. The results indicated that the basal dendritic length of L-PSP pyramidal neurons was significantly reduced 12 hr after ischemia but that the apical dendritic length of L-PSP pyramidal neurons dramatically increased 24 hr after ischemia. The soma of these cells shrank 12 hr after ischemia. In contrast, the dendritic arborization of non-L-PSP pyramidal neurons underwent only slight changes, but the soma of this subtype of neurons was persistently swollen after ischemia. These results indicate that the morphological changes of CA1 pyramidal neurons in response to transient global ischemia are heterogeneous, which might be relevant to the different pathological process of neuronal death after ischemia.

MATERIALS AND METHODS

Adult male Wistar rats (12–14 weeks old and 200–300 g weight; Charles River, Wilmington, MA) were used in the present study. Experimental protocols have been approved by the Institutional Animal Care and Use Committee (IACUC) of Indiana University School of Medicine in accordance with the NIH Guide for the care and use of laboratory animals (NIH Publications 80–230). All efforts were made to minimize the suffering and the number of animals used. In this experiment, 30 rats were used in total, nine in the control group, five in the group at 12 hr after ischemia (Is 12 h), seven in the group at 24 hr after ischemia (Is 24 h), and nine in the group at 48 hr after ischemia (Is 48 h).

Transient Forebrain Ischemia

Transient forebrain ischemia was induced by using the four-vessel occlusion method (Pulsinelli and Brierley, 1979), with modifications (Xu et al., 1999). Briefly, the animals were fasted overnight (8–12 hr) to produce uniform blood glucose levels. For the surgical procedure, the animals were anesthetized with 1–2% halothane mixed with 33% O₂ and 66% N₂. An occlusion device (a silicone tube) was placed loosely around each carotid artery to allow subsequent occlusion of the vessels. The animals were then placed in a stereotaxic frame, and the vertebral arteries were electrocauterized. A small temperature probe (0.8 mm OD) was inserted beneath the skull in the epidural space, and the brain temperature was maintained at 37°C with a heating lamp using a temperature control system (Physitemp BAT-10, Clifton, NJ). A microelectrode filled with 2 M NaCl was inserted into the hippocampus (2.5 mm below the brain surface) to record ischemic depolarization with an amplifier (Neuroprobe 1600; A-M System, Carlsborg, MA). Ischemic depolarization is the shift of extracellular DC potential from zero to approximately −20 mV after ischemia. The duration of ischemic depolarization was determined by measuring the period from the beginning of the extracellular direct current potential reaching approximately −20 mV to the point where the potential started to repolarize after recirculation. Transient forebrain ischemia was produced by occluding both common carotid arteries to induce ischemic depolarization for approximately 12 min.

Intracellular Recording and Staining In Vivo

Intracellular recording and staining in vivo were performed as described in previous publications (Xi and Xu, 1996; Gao et al., 1998). In brief, under 1–2% halothane anesthesia, the skull was opened to expose the recording site and to place the stimulating electrodes. Recording electrodes were pulled with a Kopf pipette puller (model 750; David Kopf Instrument, Tujunga, CA) from glass capillaries with a filament. Tip resistance ranged from 50 to 80 MΩ when filled with a solution of 5% neurobiotin (Vector, Burlingame, CA) in 2 M potassium acetate. Bipolar stimulating electrodes (1 mm apart) were placed into the contralateral commissural pathway (AP: 3.7–4.7 mm, ML: 1.5 mm, DV: 3.5 mm). At the placement of the recording electrode in the cortex above the hippocampus, the exposed surface of the brain was covered with soft paraffin wax. A stimulator (Master-8, A.M.P.I.) with a stimulus isolation unit (Isoflex, A.M.P.I.) was used to deliver stimulus pulses. The microelectrode was slowly advanced with a motorized micromotion controller (model EPS300; Newport Corporation, Irvine, CA) into the hippocampus at 2-μm increments to impale CA1 pyramidal neurons. The stratum pyramidale was identified by stereotaxic coordinates. After impalement, the neurons with a stable membrane potential of at least −60 mV and action potential overshoot were selected for further recording. After each successful recording, 5% neurobiotin was iontophoresed into the cells by applying rectangular depolarizing current pulse (2 Hz, 300 msec, 1.0–1.5 nA) for 15–20 min.

Tissue Preparation

The animals were sacrificed while under deep anesthesia and transcardially perfused with 0.01 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde at different intervals after ischemia. The brain was removed and stored in the same fixative overnight. The coronal sections (50 or 80 μm) were cut on the tissue blocks containing the hippocampus with a vibratome. The sections were incubated in 0.1% horseradish peroxidase-conjugated avidin D (Vector) in 0.01 M potassium phosphate-buffered saline (KPBS; pH 7.4) with 0.5% Triton X-100 overnight at room temperature. After the reaction in the solution containing 0.05% 3,3’-diaminobenzidine tetrahydrochloride (DAB) in 0.01 M PBS, the sections were examined under a microscope. The sections containing labeled cells were further processed for embedding. Two embedding protocols were used in the present study: regular embedding (the conventional process for light micros-
copy) and plastic embedding. The materials for the present study were collected over the last 7 years. The regular embedding was used in the initial studies. After we noticed considerable tissue shrinkage in regular embedding, plastic embedding was employed instead. For the regular embedding, sections (50 μm) were air dried, dehydrated, and mounted on gelatin-coated slides with low-viscosity mounting medium (Cytoseal 60; Fisher Scientific, Pittsburgh, PA). For the plastic embedding, sections (80 μm) were treated with 0.25% osmium tetroxide for 1 hr to intensify staining. Then, the sections were dehydrated, infiltrated with propylene oxide, and embedded in Epon 812 (EMS, Fort Washington, PA) as described previously (Zeng and Xu, 2000; Ruan et al., 2003, 2006).

Tissue Shrinkage Correction

Tissue shrinkage was corrected as described previously (Ruan et al., 2006). Briefly, the structural landmarks at X, Y, Z planes were made and were measured in the same sections before and after embedding procedures. The distance between two fasciculi retroflexus was as the X-plane value, and the distance between the top and the bottom of the third ventricle was as the Y-plane value. These values were measured with a ×4 objective lens. The thickness of the section (Z-plane value) from the surface to the bottom was measured with a ×60 objective lens. The correction factors were obtained by calculating the parameters before and after embeddings and employed for the sections as follows: correction factor 1.1 for X and Y planes and 4.0 for Z plane to regular embedding sections; correction factor 1.1 for all planes to the plastic embedding sections.

Three-Dimensional Reconstruction

The criteria used to select the neurons for reconstruction were as described in a previous study (Ruan et al., 2006). Briefly, all dendritic arbors of the neurons could be followed in a series of sections without abrupt truncation, and dendritic spines could be clearly identified. Only a single neuron was located within the viewing area. The drawn neurons had lost their identifiable configuration and showed very condensed debris at 7 days after ischemia (Fig. 1G,H). Almost all CA1 pyramidal neurons had lost their identifiable configuration and showed very condensed debris at 7 days after ischemia (Fig. 1I,J). Forty-four CA1 pyramidal neurons from 30 rats were selected for dendrite reconstruction based on the criteria described in Materials and Methods. The neurons were classified based mainly on the electrophysiological features (Gao and Xu, 1996; Fan et al., 2005). Consistently with our previous observations in the normal CA1 area (Fan et al., 2005), L-PSP neurons were characterized with a late depolarizing postsynaptic potential (L-PSP) in addition to an initial excitatory postsynaptic potential (EPSP) and had more branching arbors in the apical dendrites (Fig. 2A), but non-L-PSP neurons did not show a late depolarizing postsynaptic potential and have fewer branching arbors in the apical dendrites (Fig. 2B). The distribution of stained pyramidal neurons in the CA1 area was 14 in the control group (seven L-PSP neurons, seven non-L-PSP neurons), 10 in the Is 12 hr group (six L-PSP neurons, four non-L-PSP neurons), 10 in the Is 24 hr group (five L-PSP neurons, five non-L-PSP neurons), and 10 in the Is 48 hr group (four L-PSP neurons, six non-L-PSP neurons; Fig. 3A). The organization of dendritic trees in the two subtypes of pyramidal neurons in the controls was consistent with previous studies (Ishizuka et al., 1995; Pyapali et al., 1998; Ruan et al., 2006). The cell bodies were located in the stratum pyramidale. The basal dendrites bifurcated in the stratum oriens, with some distal branches reaching to the stratum alveus. The apical dendrites gave off oblique branches in stratum radiatum and stratum lacunosum/moleculare, with some distal segments extending to the hippocampal fissure (Fig. 3B). The territories of basal and apical dendritic trees were clearly identifiable in the two subtypes of the pyramidal neurons in the control group (Fig. 4A,B). However, the branching orientation of these neurons became distorted after ischemia, and this change became more obvious in L-PSP neurons than in non-L-PSP neurons (Fig. 4A,B).

Statistical Analysis

The morphometry of the dendrites and soma were exported via NeuroExplorer and were analyzed in StatView 5.0 via unpaired t-test (SAS Institute, Cary, NC). Values are presented as mean ± SE. Differences were considered significant at P < 0.05.

RESULTS

In the present study, we investigated the neuronal death at different intervals after reperfusion. The results was consistent with previous studies (Pulsinelli et al., 1982; Kirino and Sano, 1984; Crain et al., 1988). The appearance of CA1 pyramidal neurons did not change until 48 hr after ischemia (Fig. 1A–D). At 48 hr after reperfusion, a few cells had become condensed and shrunken (Fig. 1E,F, arrow), and many CA1 pyramidal neurons have similar changes at 3 days after ischemia (Fig. 1G,H, arrow). Almost all CA1 pyramidal neurons had lost their identifiable configuration and showed very condensed debris at 7 days after ischemia (Fig. 1I,J).

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Changes in Dendritic Length in the Two Subtypes of Pyramidal Neurons After Ischemia

Dendritic length is an important parameter for evaluating dendritic plasticity after ischemia. To analyze quantitatively the alteration of dendritic arborization in both L-PSP and non-L-PSP neurons in the CA1 region after ischemia, the total dendritic length (TDL), the basal dendritic length (BDL), and the apical dendritic length (ADL) were analyzed in two subtypes of neurons by crossing the control group and the ischemic groups at different intervals. As shown in Figure 5A, the TDL of L-PSP neurons markedly decreased from 17,867.85 ± 589.52 μm of control to 15,166.67 ± 1,014.47 μm.
Fig. 1. Photomicrographs showing H-E staining of sections of the hippocampus at different time points following reperfusion. The appearance of CA1 pyramidal neurons does not change until 48 hr after ischemia (A–D). A few cells become condensed and shrunken at 48 hr after reperfusion (E,F, arrow), and many CA1 pyramidal neurons have similar changes at 3 days after ischemia (G,H, arrow). Almost all CA1 pyramidal neurons lost an identifiable configuration and showed very condensed debris at 7 days after ischemia (I,J).
Factors Contributing to the Changes in Dendritic Length

Generally, there are two factors affecting dendritic length; one is the number of dendrites, and another is dendritic extension or retraction. To examine further what contributed to the changes in the dendritic length after ischemia, the number of dendrite endings, the internode interval (INI), and the dendrite terminal length (TermL) were compared in both subtypes of neurons. The numbers of the dendrite endings reflected whether the dendrites sprouted or not, whereas INI and TermL indicated whether the intermediate segments and terminal segments of the dendrites extended or retracted. Figure 6 showed the normalized changes in these three parameters after ischemia. For the basal dendrites of L-PSP neurons, the TermL was decreased by 20% (a significant decrease; Table I), the number of dendrite endings and INI also decreased by 12% and 11% compared with the controls at 12 hr after ischemia (Fig. 6A). The effect of these changes produced a significant decrease in the BDL at this time point (Fig. 5A). The values of these parameters gradually returned to controls even with a 22% higher level of INI at 48 hr after ischemia, but this did not significantly affect the value of BDL at this interval (Fig. 5A). In contrast, the number of apical dendrite endings of L-PSP neurons increased by 25% at 12 hr after ischemia, but which neutralized the declines of INI (12%) and TermL (12%) of the apical dendritic trees (Fig. 6A). Therefore, the effect of these changes did not alter the value of ADL with this episode (Fig. 5A). However, the number of apical dendrite endings dramatically increased by 42% (an significant increase; Table I) at 24 hr after ischemia, which significantly increased ADL even though the value of INI just went back to the control values and the value of TermL was still lower than the control at this time (Fig. 5A). At 48 hr after ischemia, the value of INI rose beyond 26%, but the number of dendrite endings dropped to a level 15% higher than controls, and the value of TermL returned to control values. Therefore, the whole effect of these changes only slightly increased the value of ADL at this time point (Fig. 5A). Compared with L-PSP neurons, the basal dendrite trees of non-L-PSP neurons showed almost no change in the number of dendrite endings and TermL at each time point after ischemia (Table I). However, the values of INI were obviously down (by 19%), up (by 22%), and down (by 24%) in three continuous episodes after ischemia (Fig. 6B). However, these events induced only mild changes in BDL at each time point after ischemia (Fig. 5B). For the apical dendritic trees of non-L-PSP neurons, the number of dendrite endings did not change, but INI and TermL declined slightly to 9% and 7% at 12 hr after ischemia (Fig. 6B). The number of dendrite endings moderately increased by 24%, and both INI and TermL also returned to the control levels at 24 hr after ischemia. The total effects on ADL were only moderate in this episode (Fig. 5B). At 48 hr after ischemia, the number of dendrite endings dropped to a level 10% higher than control, but both INI and TermL rose beyond 29% and 28%, which also brought out a moderate affect on ADL at this point after ischemia (Fig. 5B).
whether the dendrites spouted or extended or retracted, but where did these changes take place and what was the spatial profile of dendrites after ischemia? To answer these questions, we employed the Sholl analysis in NeuroExplorer to count the number of dendritic intersections at the different path lengths of the basal and apical dendritic trees. The unit of radius was 10 μm. For L-PSP neurons, the Sholl plots in the basal dendritic trees were almost similar before and after ischemia except that the middle part (60–160 μm from soma) was lower than control at 12 hr after ischemia (Fig. 7A). The number in this part significantly decreased from 30.92 ± 1.16 of control to 23.94 (P < 0.01; Table II). In contrast, the Sholl plots of the apical dendritic trees were obviously different in each group after ischemia (Fig. 7B). At 12 hr after ischemia, the number of intersections in the proximal part (10–100 μm from soma) markedly increased from 7.74 ± 0.99 of control to 14.21 ± 1.93 (P < 0.01) but decreased in the middle part (110–390 μm) from 15.35 ± 0.55 to 13.62 (P < 0.05) and in the distal part (400–700 μm) from 5.93 ± 0.44 to 4.53 (P < 0.05; Table II). The outcome of these changes moderately increased the number of apical dendritic trees (Table I). At 24 hr after ischemia, the number of intersections in the proximal part (10–100 μm from soma) markedly increased from 7.74 ± 0.99 of control to 14.21 ± 1.93 (P < 0.01) but decreased in the middle part (110–390 μm) from 15.35 ± 0.55 to 13.62 (P < 0.05) and in the distal part (400–700 μm) from 5.93 ± 0.44 to 4.53 (P < 0.05; Table II). The outcome of these changes moderately increased the number of apical dendritic trees (Table I). At 24 hr after ischemia, the number of intersections in the proximal and the distal parts went back almost to the control levels, whereas the number of intersections in the middle part dramatically increased to 20.51 ± 0.72 (P < 0.01; Table II), which paralleled the changes in the number of apical dendrite endings and the value of the apical dendritic length (Table I, Figs. 5A, 7B). However, the number of intersections in this part significantly decreased again to 13.22 ± 0.59 (P < 0.01) at 48 hr after ischemia (Table II). Contrasting with L-PSP neurons, the Sholl plots in the basal dendritic trees of non-L-PSP neurons were almost the same before and after ischemia (Fig. 7C). The Sholl plots of apical dendritic trees and the number of intersections were also similar before and after ischemia (Fig. 7D, Table II), but the number of intersections markedly decreased from 13.73 ± 0.57 of control to 9.97 ± 0.47 (P < 0.01) in the middle part and significantly increased from 5.81 ± 0.43 to 7.67 in the distal part (P < 0.05) at 12 hr after ischemia (Table II). However, these dynamic changes did not affect the number of apical dendritic trees in this episode (Table I).

Changes of the Somatic Size of Two Subtypes of Pyramidal Neurons After Ischemia

In the present study, we also analyzed the somatic size of two subtypes of pyramidal neurons before and after ischemia, because this might reflect the different pathological processes after ischemia. To reveal the changes in somatic size, the somatic areas in a total of 75 cell bodies were analyzed. The distribution of these cells in each group is displayed in Table III. The somatic area of L-PSP neurons was markedly reduced from 177.69 ± 11.33 μm² (n = 10) of control to 123.14 ± 7.34 μm² (n = 10; P < 0.001) at 12 hr after ischemia and became slightly larger at 24 hr (n = 12) and 48 hr (n = 10) after ischemia. However, the somatic size of non-L-PSP neurons dramatically and persistently enlarged after ischemia, from 145.29 ± 7.45 μm² (n = 10) of control to 202.28
DISCUSSION

Results in the present study revealed heterogeneous changes of CA1 pyramidal neurons in dendritic arborization and somatic size in response to transient global ischemia. This plasticity suggests that these neurons might undergo different pathological processes of neuronal death after ischemia.

Different Morphological Changes of CA1 Pyramidal Neurons in Response to Ischemia

In the present study, we have found that the morphological plasticity to ischemia is different between L-PSP neurons and non-L-PSP neurons. First, the changes of dendritic arborization are more dramatic in L-PSP neurons than in non-L-PSP neurons. Secondly, the mode of alteration between the basal dendrites and the apical dendrites is different. Dendritic retraction or extension is the major mode for the changes of the basal dendrites in two subtypes of neurons after ischemia. However, the major mode for the changes of the apical dendrites is different between the two subtypes of neurons. Dendritic sprouting is an overwhelming mode in
L-PSP neurons and determines the ADL. However, dendritic sprouting and dendritic extension or retraction almost plays a coeffective role in determining the ADL in non-L-PSP neurons. Third, the alteration of the dendritic arborization is more dynamic in L-PSP neurons than in non-L-PSP neurons, and the most changeable location is in the middle part of the apical dendritic trees in L-PSP neurons. Finally, the changing pattern of soma volume also varies in the two subtypes of cells. The soma of L-PSP neurons undergoes transient shrinkage in the early episode after ischemia, whereas that of the non-L-PSP neurons appears to swell persistently 12 hr after ischemia.

Compared with acute ischemia in the present study, chronic hypoxia also induces different changes in CA1 pyramidal neurons between the basal and the apical dendrites. It has been reported that chronic hypoxia induces retraction of the basal dendritic trees in the early stage (Pokorny et al., 1982) but sprouting in the apical dendritic trees in the later stage (Pokorny and Trojan, 1983). However, the opposite alteration in the basal dendrites and apical dendrites has been reported for the cortical pyramidal neurons (Biernaskie and Corbett, 2001). In the Biernaskie and Corbett study, the basal dendritic trees outgrow but the apical dendritic trees do not change in the 4–9 weeks after focal ischemia. These results reflect the heterogeneity of dendritic plasticity in neurons in response to hypoxia or ischemia.

Our previous studies indicate that the amplitude of initial EPSP and the L-PSP amplitude are enhanced after ischemia and reach a peak value at 24 hr after ischemia and that the duration of L-PSP is also significantly increased in this episode compared with the controls (Gao and Xu, 1996; Xu et al., 1999; Fan et al., 2005). These electrophysiological changes in L-PSP neurons might be related to the increase in the number of apical dendrites in L-PSP neurons at this time point after ischemia.

Possible Mechanisms for the Heterogeneity of CA1 Pyramidal Neurons in Response to Ischemia

Several mechanisms might be involved in the morphological changes of CA1 pyramidal neurons after
ischemia. The most important is the mechanism underlying glutamate excitotoxicity (Benveniste et al., 1984; Mitani and Kataoka, 1991; Silver and Erecinska, 1992; Lobner and Lipton, 1993; Lee et al., 2000). Generally, both AMPA and NMDA receptors mediate dendritic outgrowth and differentiation, but their effect on the dendrite growth is different. Activation of AMPA receptors induces the dendrites retraction (Metzger et al., 1998; Monnerie et al., 2003; Passafaro et al., 2003), whereas activation of NMDA receptor promotes dendrites to outgrowth (Kalb, 1994; Ikegaya et al., 2002; Sin et al., 2002). Therefore, we consider that the dra-

Fig. 6. Normalized dendritic parameters of L-PSP and non-L-PSP neurons. A: The number of dendrite endings, the INI, and the TermL in the basal dendrites of L-PSP neurons decrease at 12 hr after ischemia but gradually recover until 48 hr, with an even higher level of INI at 48 hr after ischemia. The number of endings in the apical dendrites of L-PSP neurons increases after ischemia, with a peak at 24 hr after ischemia, whereas the INI and TermL first decrease at 12 hr but gradually return to control levels, and with a higher level of INI at 48 hr after ischemia. B: The number of dendrite endings and the TermL in the basal dendrite trees of non-L-PSP neurons do not change much at each time point after ischemia. However, the values of INI display a down-up-down pattern at the continuous episodes after ischemia. For the apical dendritic trees of non-L-PSP neurons, the number of dendrite endings does not change at 12 hr but slightly increases at 24 and 48 hr after ischemia, whereas the INI and TermL first decrease at 12 hr but return to controls at 24 hr and are higher than control at 48 hr after ischemia.

| TABLE I. Changes of the Dendrite Parameters in L-PSP Neurons and non-L-PSP Neurons |
|----------------------------------------|----------------|--------|
|                                      | Basal          | Apical |
| L—PSP                                | Ends  | INI  | TermL  | Ends  | INI  | TermL  |
| Control                              | 41.43 | ± 3.84 | 48.19 | ± 3.96 | 150.22 | ± 8.38 |
| IS12h                                | 34.83 | ± 4.73 | 43.15 | ± 3.44 | 121.33 | ± 6.55* |
| IS24h                                | 38.20 | ± 5.93 | 45.45 | ± 6.36 | 158.46 | ± 13.95 |
| IS48h                                | 42.25 | ± 2.46 | 60.28 | ± 5.19 | 156.31 | ± 12.28 |
| Non-L—PSP                            | 47.60 | ± 3.97 | 56.80 | ± 5.92 | 133.92 | ± 7.78 |
| Control                              | 41.75 | ± 4.42 | 46.35 | ± 4.46 | 146.02 | ± 7.59 |
| IS12h                                | 44.75 | ± 9.41 | 69.44 | ± 9.78 | 131.79 | ± 8.58 |
| IS48h                                | 45.83 | ± 8.36 | 43.55 | ± 3.08 | 138.95 | ± 9.47 |

*P < 0.05 compared with controls.
matic dendrite sprouting in the apical dendritic trees of L-PSP pyramidal neurons at 24 hr after ischemia probably is induced by over activation of the N-methyl-D-aspartate (NMDA) receptors. A few findings are consistent with this hypothesis: First, it has reported that overexpression of NMDA receptors takes place in the

![Fig. 7. Sholl plots showing changes in the numbers of dendritic intersections in the two subtypes of neurons. A: Sholl plots in the basal dendritic trees in L-PSP neurons are almost similar before and after ischemia, except for the middle part (60–160 μm from soma), which is lower than the controls at 12 hr after ischemia. In contrast, there were large changes in the Sholl plots of the apical dendritic trees after ischemia. The plot in the middle part of the apical dendritic trees is obviously higher than that in the control group at 24 hr after ischemia (B). However, the Sholl plots of the basal dendritic trees as well as the apical dendritic trees in non-L-PSP neurons are almost the same before and after ischemia (C,D).](image)

**TABLE II. Distribution of Dendritic Intersections in CA1 Pyramidal Neurons (Number/10 μm)**

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th></th>
<th>Apical</th>
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<tr>
<td></td>
<td>10–50 μm</td>
<td>60–160 μm</td>
<td>170–350 μm</td>
<td>10–100 μm</td>
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<tr>
<td>L—PSP</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>10.74 ± 1.21</td>
<td>30.92 ± 1.51</td>
<td>10.13 ± 1.41</td>
<td>7.74 ± 0.99</td>
</tr>
<tr>
<td>IS12h</td>
<td>9.13 ± 1.14</td>
<td>23.94 ± 1.16**</td>
<td>8.10 ± 1.13</td>
<td>14.21 ± 1.93**</td>
</tr>
<tr>
<td>IS24h</td>
<td>11.83 ± 1.30</td>
<td>30.47 ± 0.91</td>
<td>8.09 ± 1.10</td>
<td>9.87 ± 0.94</td>
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<tr>
<td>IS48h</td>
<td>16.10 ± 2.92</td>
<td>33.50 ± 2.16</td>
<td>5.74 ± 1.24*</td>
<td>7.77 ± 1.14</td>
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<tr>
<td>Non-L—PSP</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>12.71 ± 1.29</td>
<td>30.72 ± 1.06</td>
<td>9.61 ± 1.26</td>
<td>8.22 ± 0.85</td>
</tr>
<tr>
<td>IS12h</td>
<td>11.15 ± 1.67</td>
<td>31.77 ± 1.13</td>
<td>7.06 ± 1.23</td>
<td>7.10 ± 0.98</td>
</tr>
<tr>
<td>IS24h</td>
<td>12.75 ± 2.66</td>
<td>32.82 ± 2.33</td>
<td>10.87 ± 1.63</td>
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<tr>
<td>IS48h</td>
<td>10.87 ± 1.42</td>
<td>30.48 ± 1.69</td>
<td>8.89 ± 1.20</td>
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*P < 0.05 compared with controls.
**P < 0.01 compared with controls.
Heterogeneity of Morphological Alteration and Variety of Neuronal Death in CA1 Pyramidal Neurons After Ischemia

It has been reported that more than 95% of CA1 pyramidal neurons will die 3 days later after ischemia (Pulsinelli et al., 1982), which suggests that most of the CA1 pyramidal neurons labeled in the present study will die later after reperfusion. Therefore, the morphological plasticity of CA1 pyramidal neurons in the early stage of delay neuronal death most likely links to neurodegeneration in the CA1 area even though we cannot exclude the possibility of compensatory mechanisms. Previous studies have shown the different modes of neuronal death in the CA1 area and striatum after ischemia, including apoptosis, necrosis, or a mixture of apoptosis and necrosis (Nitatori et al., 1995; Choi, 1996; Colbourne et al., 1999; Zeng and Xu, 2000; Ruan et al., 2003). In the present study, the heterogeneity of morphological alterations to CA1 pyramidal neurons within 2 days after ischemia might be associated with the diversity of neuronal death in the CA1 region. The fates of neurons after ischemia may be determined by the distribution of NMDA and non-NMDA receptors in these cells. It has been reported that application of NMDA receptor agonists induces necrosis with a swollen cell body, whereas non-NMDA receptor agonists trigger apoptosis with shrinkage of soma and nucleus (Portera-Cailliau et al., 1997a,b). Therefore, we consider that L-PSP neurons might involve both apoptotic and necrotic pathways because of their soma displaying early shrinkage but later enlargement, whereas non-L-PSP neurons might go into the necrotic pathway because of their soma showing persistent swelling after ischemia.

ACKNOWLEDGMENTS

The authors thank Miss Mandy Gacsko and Dr. Michael Kubek for proofreading the manuscript.

REFERENCES


