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CA3 NEURONAL ACTIVITIES OF DORSAL AND VENTRAL HIPPOCAMPUS ARE DIFFERENTIALLY ALTERED IN RATS AFTER PROLONGED POST-ISCHEMIC SURVIVAL

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Abstract—The aim of the present study is to explore the potential hyper-excitability of hippocampal CA3 neurons in rats after prolonged post-ischemic survival. We conducted 15-min four-vessel-occlusion ischemic episodes in rats, allowed these animals to survive for approximately 8 months and then examined the basic morphological features and population synaptic activities of CA3 neurons. In fixed tissue sections obtained from dorsal hippocampi of post-ischemic rats, we observed a complete loss of the CA1 neurons together with a shrunken CA1 sector. Extracellular recordings in slices revealed that the overall synaptic activities of dorsal hippocampal CA3 neurons were decreased in post-ischemic rats compared with sham-operated controls. Both sham control and post-ischemic ventral hippocampal neurons were capable of exhibiting intermittent spontaneous field potentials in slices. These spontaneous field potentials spread from the CA3 to the CA1 area and their generation relied on the activity of glutamate alpha-amino-3-hydroxy-5-methyl-4 isoxazole proprionic acid (AMPA) receptors. The propensity for displaying these spontaneous field potentials appeared to be greater in post-ischemic slices than sham control slices. Our data suggest that the hyper-excitability of the post-ischemic hippocampus, if it occurs, may preferentially take place in the ventral CA3 circuitry.

Key words: neuronal network, oscillation, seizure, slices, spontaneous, stroke.

In humans and experimental animals, the hippocampus is highly vulnerable to transient brain ischemia. A majority of CA1 pyramidal neurons of the dorsal hippocampus in rat models of transient forebrain ischemia are irreversibly injured after several days of reperfusion, while CA3 pyramidal neurons that project to the CA1 area remain morphologically intact during this period (Pulsinelli and Brierley, 1979; Smith et al., 1984). Although morphologically identified CA3 neuronal injuries have been observed in rats after ≥1 month of post-ischemic survival (Onodera et al., 1990; Nunn et al., 1994; Nelson et al., 1997; Modo et al., 2000), only limited information is available thus far regarding the electrophysiological properties of post-ischemic CA3 neurons. CA3 neuronal networks are thought to play a crucial role in memory information processes (McNaughton and Morris, 1987; Hasselmo and Wyble, 1997; Lisman, 1999; Lorincz and Buzsáki, 2000; Nakazawa et al., 2002, 2003) and in the generation of synchronous neuronal activities (Miles and Wong, 1987; Traub et al., 1989). Therefore, further study of post-ischemic CA3 neurons will help to develop our understanding of the post-ischemic recovery of hippocampus-dependent cognitive functions (Block, 1999) and to explore potential mechanisms of post-ischemic seizures (Camilo and Goldstein, 2004).

Congar et al. (2000) have examined intracellular and synaptic activities of post-ischemic CA3 neurons *in vitro* via conducting 20–25 min of four-vessel occlusion (FVO) ischemic episodes in rats and preparing hippocampal slices from these animals after 2–4 months of post-ischemic survival. They demonstrated that resting membrane potentials were approximately 5 mV more positive in post-ischemic CA3 pyramidal neurons than in sham controls. Moreover, slices prepared from both sham control and post-ischemic rats did not exhibit spontaneous field potentials, but the post-ischemic slices were more susceptible to displaying self-sustained or evoked bursting field potentials after treatments with high K^+ or kainate. Congar et al. (2000) suggested that post-ischemic CA3 pyramidal neurons have a reduced threshold for generating seizure-like activities.

The primary aim of the present study is to examine CA3 population synaptic activities in rats that experience less severe ischemic episodes but longer post-ischemic survival as compared with Congar et al.'s (2000) model. Since the ventral hippocampus is less vulnerable to transient ischemia (Ashton et al., 1989; Auer et al., 1989; Rami et al., 1997) but more susceptible to displaying epileptiform activities (Racine et al., 1977; Gilbert et al., 1985; Borck and Jefferys, 1999; Derchansky et al., 2004) than the dorsal hippocampus, we questioned whether hyper-excitable population activities occur spontaneously in the post-ischemic ventral hippocampal circuitry.

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Abbreviations: ACSF, artificial cerebrospinal fluid; AMPA, alpha-amino-3-hydroxy-5-methyl-4 isoxazole proprionic acid; BIFP, bicuculline-induced field potential; DG, dentate gyrus; EEG, electroencephalography; EPSPs, excitatory postsynaptic potentials; FVO, four-vessel occlusion; GABA, gamma-aminobutyric acid; IPSPs, inhibitory postsynaptic potentials; NMDA, *N*-methyl-D-aspartate; SFP, spontaneous field potential; 4-AP, 4-aminopyridine.

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We conducted a 15-min FVO in rats, allowed these animals to survive for approximately 8 months post-ischemia and then examined the morphological features and synaptic activities of hippocampal neurons in slices. We found that the overall synaptic activities of CA3 neurons of the post-ischemic dorsal hippocampus were attenuated as compared with sham controls, whereas the post-ischemic ventral hippocampus appeared to have a high propensity for exhibiting spontaneous field potentials that originated from the CA3 area and were dependent upon the glutamatergic activity mediated by alpha-amino-3-hydroxy-5-methyl-4 isoxazole proprionic acid (AMPA) receptors.

EXPERIMENTAL PROCEDURES

Transient forebrain ischemia

Male Wistar rats (Charles River, Canada, Saint-Constant, Quebec, Canada) were used in the present experiments. All experimentation conducted in this study has been reviewed and approved by the animal care committee of University Health Network, according to the standard operating procedures that are conformed to the requirements of the Animals for Research Act and the Canadian Council on Animal Care Guidelines. Only a minimal and necessary number of animals were used in this study. Care was taken to avoid unnecessary suffering or pain of the animals during our experimentation. These rats weighed 180–200 g while they received sham surgery or ischemic episodes. Transient forebrain ischemia was conducted as previously described (Shinno et al., 1997; Zhang et al., 1997, 1999; Hsu et al., 1998; Francis et al., 1999; Shen et al., 2002) using a modified version of the FVO model as per Pulsinelli and Brierley (1979). Briefly, the rat was anesthetized with halothane (2% in a 2:1 nitrous oxide–oxygen mixture) on day 1 and electrocauterization of vertebral arteries was performed. On day 2, under halothane anesthesia, the carotid arteries were clamped with aneurysm clips for 15 min. Rectal temperatures were kept at 36.5–37 °C throughout the experimental period via a warming lamp. The animals were selected for further experimentation if they showed 1) an isoelectric electroencephalographic (EEG) signal during the occlusion period and recovered EEG activities within 30 min after termination of the carotid artery occlusion; 2) pupil dilation and no corneal reflex during the occlusion; and 3) stable rectal temperatures. Sham rats were similarly operated but without artery occlusion. The animals were closely monitored in our laboratory for several hours after termination of anesthesia and then returned to our animal facility where they were examined two to three times daily for 2–3 days and once a week afterward. We did not observe behavioral seizures and neurological deficits in these post-ischemic rats. However, we could not rule out the possibility that localized or electrographic seizures might occur without motor manifestation. All animals were housed individually in a temperature-controlled room (22–23 °C) with a 12-h light/dark cycle. Food and water were freely accessible before and after operation. The animals were allowed to survive 7.5–8 months after FVO or sham surgery before electrophysiological and morphological assessments.

Electrophysiological recordings in brain slices

For the preparation of brain slices, we anesthetized the rat via a mixture of ketamine (25 mg/ml), xylazine (1.3 mg/ml) and acepromazine (0.25 mg/ml; 4 ml per kg, i.p., Mody et al., 1995) and then carried out intra-cardiac infusion with a cold (approximately 4 °C) artificial cerebrospinal fluid (ACSF). The brain was then quickly removed, hemi-sectioned and maintained in an ice-cold, oxygenated (95% O_2-5% CO_2) ACSF for 4–5 min before further manipulation. One half of the brain was then fixed with a 4% paraformaldehyde/0.1 M phosphate buffer for morphological assessments. The other half was used for preparing brain slices for electrophysiological recordings. To prepare brain slices along the transverse plane of the ventral or dorsal hippocampus, the hemi-sectioned brain was glued onto an aluminum block with its basal (ventral) side facing down and a dorsal brain chunk of 4 mm thickness was obtained via a vibrotome cut (series 1000; Technical Products International Incorporated, St. Louis, MO, USA). Horizontal brain slices (500 μm in thickness) that contained ventral hippocampal tissue were then obtained from the remaining basal brain via vibrotome cuts (three slices per half brain). The dorsal brain chunk was then glued onto an agar block with the longitudinal axis of the dorsal hippocampus aligned vertically. Vibrotome slices of 500 μm thickness that contained dorsal hippocampal tissue were cut along the transverse plane of dorsal hippocampus (three to four slices per half brain). These slices were kept in warmed (32–33 °C), oxygenated ACSF for 1–6 h before recording. The components of the ACSF were (in mM): KCl 3.5, NaH_2PO_4 1.25, NaCl 125, $NaHCO_3$ 25, $CaCl_2$ 2, $MgSO_4$ 1.3 and glucose 10. The pH of the ACSF was approximately 7.4 when aerated with 95% O_2-5% CO_2.

A custom submerged chamber was used for *in vitro* recordings (Wu et al., 2002). Briefly, the slice was held on a stainless steel fine mesh via six to eight mosquito pins. The mesh was set approximately 1.5 mm above the bottom of the chamber to allow the perfusion of the oxygenated ACSF to both sides of the slice. The oxygenated ACSF was warmed to 33–34 °C and perfused to the slice at a rate of 6–7 ml per minute. A warm bath underneath the recording chamber was set at 32 °C via an automatic temperature control unit, which allowed warmed and humidified 95% O_2-5% CO_2 passing over the perfusate to increase the local oxygen tension of the recording chamber. By placing a fine temperature probe near the perfused slices, we verified that the temperature of perfused ACSF was at approximately 32 °C.

Extracellular recording electrodes were pulled from thin-wall glass pipettes (World Precision Instruments, Sarasota, FL, USA; 1.5 mm outside diameter) via a vertical Narishige puller (Narishige Scientific Instrument Laboratory, Setagaya-ku, Tokyo, Japan). The resistance of these electrodes was approximately 2 MΩ when filled with a solution containing 150 mM NaCl and 2 mM HEPES (pH 7.4). Signals were sampled via an Axoclamp-2B or multi-clamp 700A amplifier (Axon Instruments, Union City, CA, USA) and digitized via a D/A interface (Digidata 1200 or Digidata 1300A; Axon Instruments). Data acquisition, storage and analyses were performed via PCLAMP software (version 8 or 9; Axon Instruments).

For afferent stimulation, a bipolar tungsten wire electrode was placed in the CA3 stratum radiatum and constant current pulses of 0.1 ms were generated by a Grass stimulator (S88, Grass Telefactor, West Warwick, RI, USA) and delivered via an isolation unit every 30 s. For paired afferent stimulation, an identical current pulse was repeated at an interval of 100 ms. Enhancements in synaptic potentials following the paired stimulation are expressed as the amplitude ratio of the two evoked responses, taking the first response as 100%.

Morphological examinations

The hemi-sectioned, fixed brain was cut horizontally via the vibrotome to obtain ventral and dorsal brain chunks as described above. Frozen sections (15 μm in thickness) were then obtained via cryostat along the transverse plane of the ventral or dorsal hippocampus. Because relatively small brain chunks were mounted for cryostat sections, successful section collections were made from limited ventral and dorsal hippocampal areas of 1–2 mm in length. These sections were stained with Cresyl Violet (Smith et al., 1984; Rod and Auer, 1992; Shinno et al., 1997; Wu et al., 2002) and visualized under a light microscope. Cell counts were conducted under a 40× objective lens. For each sham control and post-ischemic rat, cell count data were collected from three to five dorsal and ventral hippocampal sections. CA3 and CA1 neurons were considered injured if they