Vulnerability to Seizures Induced by Potassium Dyshomeostasis in the Hippocampus in Aged Rats

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Neurological diseases such as ischemia and dementia increase with aging, while whether seizure susceptibility increases with aging remains debatable. Seizure activity frequently originates in the hippocampus and is linked to excess of extracellular glutamate. To analyze seizure susceptibility in aged rats, the response of hippocampus excited excessively was evaluated based on extracellular concentrations of neurotransmitters and epileptic behavior. In 8-week-old and 90–100-week-old rats, which exhibited normal passive avoidance behavior, the hippocampus was stimulated with 50–100 mM KCl using *in vivo* microdialysis. The basal concentrations of extracellular glutamate and γ-amino butyric acid (GABA) were almost the same between young and aged rats. The changes in their concentrations after stimulation with 50–100 mM KCl were not also appreciably different between them. However, seizures, *i.e.*, myoclonic jerks, were observed only in aged rats during the stimulation. These results suggest that aged rats are vulnerable to seizures induced by dyshomeostasis of potassium and chloride ions in the hippocampal extracellular fluid. Homeostasis of electrolytes in the hippocampal extracellular fluid seems to be important in preventing seizure activity in the elderly.

Key words —— glutamate, seizure, hippocampus, potassium, microdialysis, aging

INTRODUCTION

The number of neurons in the brain and cerebral blood flow are decreased with aging. Aging is characterized by a progressive loss of functions and lead to a decline in cognitive functions.[1, 2] Age-related memory impairment is observed even in persons without neurological diseases.[3] Normal aging is not associated with widespread neuronal loss, but becomes increasingly vulnerable to the effects of excessive metabolic loads, usually associated with trauma, ischemia or neurodegenerative process.[4] Neurological diseases such as ischemia and dementia increase with aging. In both acute and chronic neurological diseases, glutamate excitotoxicity via excess of extracellular glutamate concentration is involved in neuronal damage.[5–7]

Extracellular glutamate is usually maintained in a low concentration and its concentration is estimated to be around 3–4 μM.[8] Glutamate can be released from the cells by two mechanisms: either by calcium-dependent vesicular release or, in pathological conditions, by reversed operation of the plasma membrane glutamate uptake carrier.[9, 10] In brain ischemia, extracellular potassium concentration increases rapidly and reaches 75 mM, followed by excess of extracellular glutamate,[11] probably via neuronal depolarization or glial swelling.[12] Excess of extracellular glutamate is also associated with epileptic seizures;[13] Seizure activity frequently originates in the hippocampus and is linked to excess of extracellular glutamate. On the other hand, extracellular glutamate are excessively increased in the hippocampus stimulated with 100 mM KCl. However, there is no report on epileptic seizures after the stimulation. All such experiments have been usually performed in young and adult animals. Examining the relationship between the increase in extracellular glutamate and epileptic seizures in association with aging is helpful to understand seizure susceptibility in the elderly.

In the present study, to analyze seizure susceptibility in aged rats, the response of hippocam-

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pus stimulated with 50–100 mM KCl was evaluated based on extracellular concentrations of neurotransmitters and epileptic behavior.

MATERIALS AND METHODS

Chemicals —— Artificial cerebrospinal fluid (ACSF) used as a perfusate was composed of 127 mM NaCl, 2.5 mM KCl, 1.3 mM $CaCl_2$, 0.9 mM $MgCl_2$, 1.2 mM Na_2HPO_4, 21 mM $NaHCO_3$ and 3.4 mM D-glucose (pH 7.3). To stimulate the hippocampus, 50–100 mM KCl was added to ACSF.

Experimental Animals —— Wistar rats were purchased from Japan SLC (Hamamatsu, Japan). They were housed under the standard laboratory conditions (23 ± 1°C, 55 ± 5% humidity) and had access to tap water and diet *ad libitum*. The lights were automatically turned on at 8:00 and off at 20:00. All experiments were performed in accordance with the Japanese Pharmacological Society guide for the care and use of laboratory animals.

Passive Avoidance Performance —— The apparatus (Step Through Test System MST-01S, Muromachi Kikai Co., Ltd., Tokyo, Japan) consists of two compartments separated by a black wall with a hole in the lower middle part. Of the two compartments, one is illuminated and the other is dark. The test was conducted for 2 days including one acquisition trial and one testing (retention) trial at the same time of the day. In the acquisition trial, a rat was put into the bright compartment for 60 sec. When the hole to enter the dark compartment was open, the rat entered the dark compartment within 60 sec, suffered an electric foot shock (3 mA) for 3 sec through the stainless steel grid floor in the dark compartment, and was put in the same compartment without the electric shock for 15 sec. After 24 hr, the retention trial was performed in the same manner; the rat was put into the bright compartment again for 60 sec. The hole to enter the dark compartment was open and the time when the rat entered the dark compartment was recorded. Rats were allowed to stay in the bright compartment for 240 sec in the retention trials.

***In vivo* Microdialysis** —— The dose of chloral hydrate used to anesthetize rats was determined based on no behavioral response to stimulating the sole with tweezers. Eight-week-old or 90–100-week-old rats were anesthetized with chloral hydrate [young rats (body weight, 208 ± 3 g), 350 mg/kg; aged rats (485 ± 17 g), 300 mg/kg] and individually placed in a stereotaxic apparatus. The skull was exposed, a burr hole was drilled, and a guide tube (CMA Microdialysis, Solna, Sweden) was implanted into the right ventral hippocampus (AP −5.6 mm, ML +4.6 mm, VD +4.1 mm). The coordinate is the values on the brain map.[14] The guide tube was secured with dental cement and screws. After the surgical operation, each rat was housed individually. Forty-eight hr after implantation of the guide tube, a microdialysis probe (3-mm membrane CMA 12 probe, CMA Microdialysis) was inserted into the ventral hippocampus of chloral hydrate-anesthetized rats through the guide tube. The hippocampus was preperfused at 5.0 μl/min with ACSF for 60 min to stabilize under anesthesia. Then the hippocampus was perfused at 5.0 μl/min with ACSF for 60 min to determine the basal concentration of neurotransmitters and perfused at 5.0 μl/min with 50 mM KCl in ACSF for 20 min and 100 mM KCl in ACSF for 20 min. The perfusate was collected every 20 min.

Young and aged rats were individually observed by two persons to determine behavioral change during hippocampal perfusion. Because myoclonic jerks were frequently observed in aged rats during perfusion with 50–100 mM KCl in ACSF, the total time (duration) measured by two persons was averaged.

The position of inserted microdialysis probe was checked after the experiment; the brains were removed from anesthetized rats and frozen at −20°C. Coronal slices were prepared from the brains and it was checked that microdialysis probes were inserted into the ventral hippocampus.

HPLC Analysis —— The perfusate samples were analyzed for glutamate aspartate, γ-amino butyric acid (GABA), glycine, taurin contents by high-performance liquid chromatography (HPLC) [column, CAPCELL PAK C18 UG120A (1 mm × 150 mm), Shiseido Co. Ltd., Tokyo, Japan; mobile phase, 0.1 M potassium dihydrogen phosphate, 0.1 M di-sodium hydrogen phosphate, 10% acetonitrile, 0.5 mM EDTA-2Na, 3% tetrahydrofuran, pH 6.0] using the pre-column derivatization technique with o-phthaldialdehyde and a fluorescence detector (NANOSPACE SI-2, Shiseido Co. Ltd.). The perfusate samples were also analyzed for serotonin and 5-hydroxyindoleacetic acid (5-HIAA), a serotonin metabolite, contents by HPLC [column, CAPCELL PAK C18 UG120 V (1.5 mm × 250 mm), Shiseido Co. Ltd.; mobile phase, 0.1 M potassium dihydrogen phosphate, 0.65 mM 1-octanesulfonic

acid sodium salt, 0.027 mM EDTA-2Na, 5% acetonitrile, pH 3.0] using an electrochemical detector (NANOSPACE SI-2/3005, Shiseido Co. Ltd.). The neurotransmitter concentrations in the perfusate samples were calculated from each peak area of the neurotransmitter standard solution, which was analyzed before and after analysis of samples.

Zinc Concentration in the Hippocampus —— The brains were excised from 8-week-old and 90–100-week-old rats under deep diethyl ether anesthesia. The hippocampus was excised from the brains, weighed, ashed at 70°C in 1.0 ml of nitric acid in glass centrifuge tubes and diluted with distilled deionized water before analysis. Zinc concentration was measured with a flameless atomic absorption spectrophotometer (Shimadzu AA6800F, Kyoto, Japan).

Timm's Sulfide-Silver Staining —— Eight-week-old or 90–100-week-old rats were anesthetized with chloral hydrate and then perfused transcardially with 0.1% Na_2S in phosphate buffer (pH 7.4). The brains were excised from the rats, frozen immediately, fixed quickly with ice-cold 4% sodium carboxymethyl cellulose, and then sliced in 30 μm thickness at −20°C with a microtome. Timm's staining was performed according to the procedure described previously.[15)]

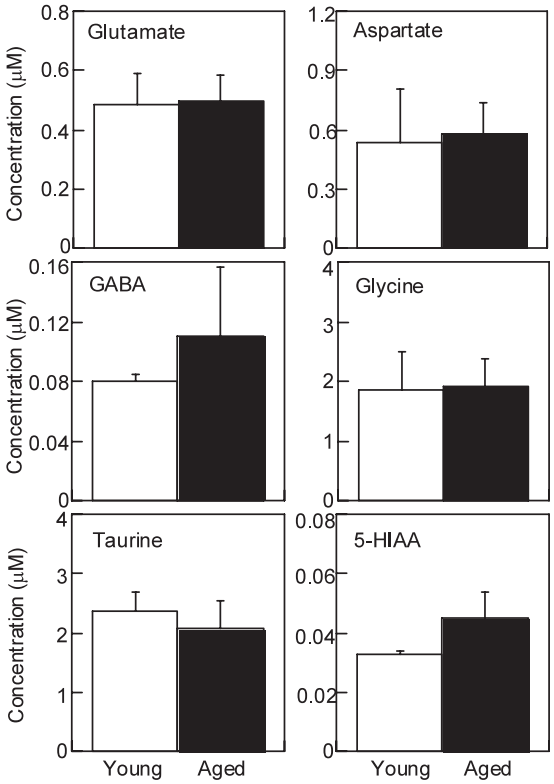

Fig. 1. Extracellular Concentrations of Glutamate, Aspartate, GABA, Glycine, Taurin and 5-HIAA in the Hippocampus

The hippocampus of young (8-week-old) and aged (90–100-week-old) rats was perfused with ACSF. Three samples corrected every 20 min were averaged. Each bar and line represents the mean ± SEM (n = 6).

RESULTS

In the passive avoidance performance, all young (8-week-old) and aged (90–100-week-old) rats tested successfully entered the dark compartment within 60 sec in the acquisition trial. None of young and aged rats entered the dark room in the retention trial, indicating that there is no difference in passive avoidance performance between young and aged rats.

To evaluate neurotransmitter system in the hippocampus of aged rats, the hippocampus was perfused using *in vivo* microdialysis. The basal concentrations of extracellular glutamate, aspartate, GABA, glycine, taurin and 5-HIAA, a serotonin metabolite, were not appreciably different between young and aged rats (Fig. 1). When the hippocampus was stimulated with 50–100 mM KCl in ACSF, the concentrations of extracellular glutamate and GABA were increased in the same manner between young and aged rats (Fig. 2A, 2B). On the other hand, there was a difference in behavioral response between them; only in aged rats, seizures, *i.e.*, myoclonic jerks, were observed during perfusion with 50–100 mM KCl in ACSF (Fig. 2C). The rate of seized aged rats was 83%. When the total time (duration) of myoclonic jerks was averaged, it was 118 sec in the observing time (40 min).

Because hippocampal zinc is associated with the etiology and manifestation of epileptic seizures,[16)] zinc concentration in the hippocampus was compared between young and aged rats. Zinc concentration of aged rats was not different from that of young rats (data not shown). Timm's staining which is used to detect histochemically reactive zinc in the presynaptic vesicles, was also performed in the hippocampus. Timm's stain was almost the same between young and aged rats; hippocampal mossy fibers were strongly stained in both groups (Fig. 3).

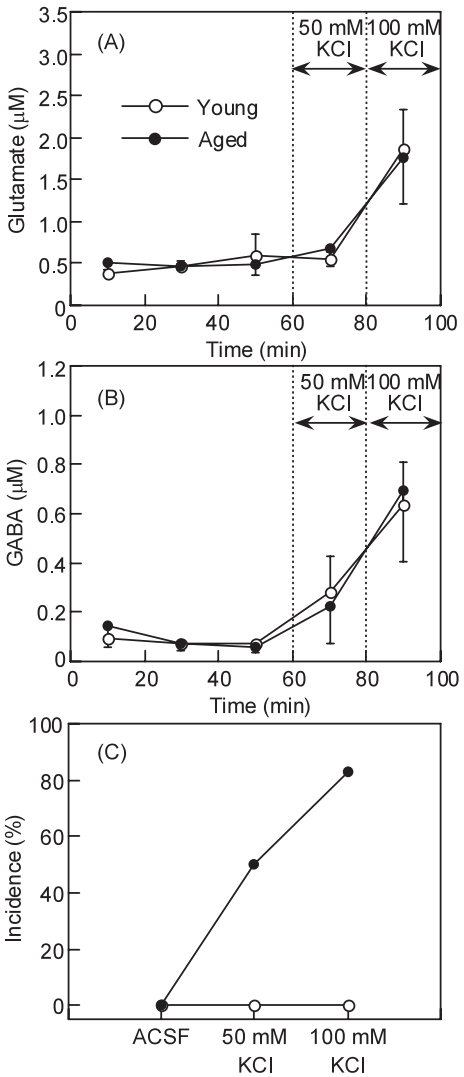

Fig. 2. Extracellular Concentrations of Glutamate and GABA in the Hippocampus after Stimulation with High [K^+]

The hippocampus of young (8-week-old) and aged (90–100-week-old) rats was perfused with ACSF for 60 min and then perfused with 50 mM KCl in ACSF for 20 min and 100 mM KCl in ACSF for 20 min. Glutamate (A) and GABA (B) concentrations in the perfusate, which were collected every 20 min, were determined by HPLC. Each bar and line represents the mean ± SEM (n = 6). The incidence (C) represents the rate of rats, in which myoclonic jerks were observed during hippocampal perfusion (n = 12).

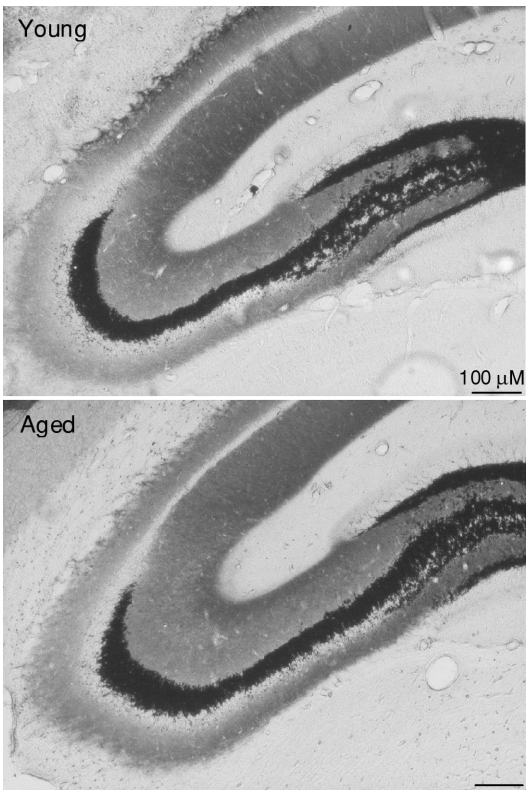

Fig. 3. Timm's Stain in the Hippocampus

Timm's sulfide-silver staining was performed to estimate zinc concentrations in synaptic vesicles. Coronal slices (30 μm thickness) for zinc staining were prepared from the brain of young (8-week-old) and aged (90–100-week-old) rats (n = 6).

DISCUSSION

Photothrombosis used to produce neocortical infarction induces seizure activity in aging rats.[17] Although the technique of photothrombosis seems to be a means to study the mechanism of secondary epileptogenesis, it is unclear whether aged rats are vulnerable to infarction-induced seizure activity. Increased susceptibility of aged individuals to neurological or epileptogenic effects of domoic acid in humans and of kainic acid in rats has been reported.[18–20] With increasing numbers of elderly persons with epilepsy needing appropriate treatment, understanding the basic mechanisms of epilepsy is crucial.[21] On the other hand, other reports show that aged rats are less susceptible to the excitotoxic action of kainic and quinolinic acids.[22–24] Whether susceptibility to epileptic seizures increases with aging remains debatable. In the present study, response to potassium dyshomeostasis in the hippocampus, which is observed in brain ischemia and elicit excess of extracellular glutamate,[11] was compared between young and aged rats.

The ability of passive avoidance was not affected in aged rats used in the present study. Early in the aging process, calcium ion homeostasis in brain cells begins to be dysregulated.[25] An increase in the calcium-dependent afterhyperpolarization is correlated to hippocampal aging.[26] The number of available L-type voltage-dependent calcium channels in hippocampal CA1 pyramidal neurons increases with aging.[27] Calcium ion homeostasis is

closely related to neurochemical response in the hippocampus. The basal concentrations of extracellular glutamate, aspartate, GABA, glycine, taurin and 5-HIAA were almost the same between young and aged rats. The increase in the concentration of extracellular glutamate and GABA after stimulation with 50–100 mM KCl was not appreciably different between them, whereas myoclonic jerks were observed only in aged rats. The increase in extracellular glutamate may trigger spontaneous seizures in patients with complex partial epilepsy.[13] In the present study, it is possible that extracellular glutamate in the hippocampus was temporarily increased in association with seizure activity in aged rats and that the increase was not detected. Although the increase in extracellular glutamate is not always correlated to seizures severity, seizure score (severity) of myoclonic jerks is low. The present study is the first to demonstrate that the increase in extracellular potassium and chloride ions in the hippocampus induces seizure activity in aged rats.

Acute and/or severe electrolyte imbalances frequently cause seizures; seizures are observed in patients with sodium disorders, hypocalcemia and hypomagnesemia, but not in patients with hypokalemia and hyperkamemia.[28] The correct diagnosis of seizures secondary to electrolyte abnormalities begins with a complete serum chemistry evaluation. Such an electrolyte screening is important in the elderly, in which metabolic disturbances, *e.g.*, hyponatremia and hypoglycemia, are common.[29,30] The concentration of brain extracellular electrolytes is similar to that of serum electrolytes. Thus, the change in serum electrolyte concentrations seems to influence the concentrations of extracellular electrolytes in the brain. Severe potassium abnormality in the serum may induce fetal arrhythmias or muscle paralysis before brain symptoms appear.[31,32] It is possible that the increase in potassium concentration in the hippocampal extracellular fluid induces seizure activity. Aged rats might be vulnerable to seizures induced by dyshomeostasis of extracellular potassium in the hippocampus.

On the other hand, seizure susceptibility of EL (epilepsy) mice is decreased by dietary zinc loading, while it is increased by dietary zinc deficiency.[33] Susceptibility to kindled seizures is also decreased by dietary zinc loading, while this susceptibility in cats is increased by zinc deficiency.[34] Zinc homeostasis in the brain is associated with the etiology and manifestation of epileptic seizures.[35] In the hippocampus, zinc exists in the terminals of mossy fibers and Schaffer collaterals; zinc exists in the presynaptic vesicles of a subclass of glutamatergic neurons[36] and is histochemically reactive. The zinc detected by Timm's staining may negatively modulate glutamate release in the hippocampus.[37] In vivo microdialysis experiments indicate that the concentration of extracellular glutamate in the hippocampus is decreased in the presence of zinc.[38] It is possible that hippocampal zinc levels are associated with seizure susceptibility in aged rats. However, no change in hippocampal zinc levels was observed. In conclusion, homeostasis of electrolytes in the hippocampal extracellular fluid seems to be important in preventing seizure activity in the elderly.

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