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IMPAIRMENT OF RECOGNITION MEMORY AND HIPPOCAMPAL LONG-TERM POTENTIATION AFTER ACUTE EXPOSURE TO CLIOQUINOL

A. TAKEDA,* S. TAKADA, M. ANDO, K. ITAGAKI, H. TAMANO, M. SUZUKI, H. IWAKI AND N. OKU

Department of Medical Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, Global COE-21, 52- 1 Yada, Surugaku, Shizuoka 422-8526, Japan

Abstract—Clioquinol (CQ) was associated with cases of transient global amnesia and with the neurodegenerative syndrome subacute myelo-optico-neuropathy (SMON) in humans. However, CQ forms lipophilic chelates with cations and has the potential as a scientific and clinical tool used for selective modulation of histochemically reactive zinc pools. The relationship among transient lack of synaptic zinc release, hippocampal long-term potentiation (LTP) induction and cognitive memory is poorly understood. To evaluate the role of synaptic zinc release, in the present study, hippocampal LTP induction and cognitive behavior were examined in young rats after i.p. injection of CQ (30 mg/kg). Intracellular zinc detected by Timm's stain and extracellular (synaptic cleft) zinc detected by ZnAF-2 were significantly decreased in the hippocampus 6 h after CQ injection. The molecular layer of the dentate gyrus, in which perforant path-granule cell synapses exist, was most responsive to CQ injection. Dentate gyrus LTP was induced similarly to the control 2 h after CQ injection, while significantly attenuated 6–24 h after CQ injection. In the training trial of the object recognition memory 2 h after CQ injection, there was no significant difference in learning behavior between the control and CQ-treated rats. In the test trial, CQ-treated rats showed normal recognition memory 1 h after the training, whereas recognition memory deficit 24 h after the training unlike the control rats. These results indicate that acute exposure to CQ impairs long-term (24 h) memory in the hippocampus of young rats. The CQ-mediated attenuation of dentate gyrus LTP, which may be associated with the transient lack of zinc release from zincergic neurons, seems to be involved in the impairment of the long-term memory.

Key words: hippocampus, clioquinol, zinc, LTP, memory.

The hippocampus plays an important role in learning, memory and recognition of novelty. The hippocampus receives major input from the entorhinal cortex via the perforant pathway. The dentate granule cells project to the CA3 pyramidal cells via the mossy fibers. The CA3 pyramidal cells project to the CA1 pyramidal cells via the Schaffer collaterals. The three pathways are glutamatergic and terminals of them are stained by Timm's sulfide-silver method, which detects histochemically reactive zinc (Frederickson, 1989; Frederickson and Danscher, 1990). The zinc predominantly exists in the presynaptic vesicles and serves as an endogenous neuromodulator (Smart et al., 1994; Takeda and Tamano, 2009). Zinc multi-functionally modulates hippocampal long-term potentiation (LTP), a widely studied model of memory; zinc attenuates mossy fiber LTP at low micromolar concentrations (Takeda et al., 2008; Ando et al., 2010), while potentiates NMDA receptor-dependent CA1 LTP (Takeda et al., 2009), unlike NMDA receptor-independent CA1 LTP (Takeda et al., 2010). However, the role of zinc in dentate gyrus LTP is unknown.

For better understanding the functions of histochemically reactive zinc and the mechanisms through which it acts, many approaches such as application of zinc chelators and gene knockouts of zinc-regulating proteins have been performed (Vogt et al., 2000; Cole et al., 2001; Li et al., 2001; Lee et al., 2002; Adlard et al., 2010). However, the relationship among transient loss of histochemically reactive zinc, hippocampal LTP induction and cognitive memory is poorly understood and is controversial. To study the role of histochemically reactive zinc in cognitive memory, a zinc-specific chelator is needed that is taken up into the brain. It is also required that the chelator does not interfere with the tightly bound zinc pool, such as zinc fingers and numerous catalytic enzymes, which are essential for cellular functions. Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline; CQ) forms lipophilic chelates with cations such as Zn^{2+} and Cu^{2+} and is a possible candidate (Nitzan et al., 2003; Andersson et al., 2009; Bareggi et al., 2009; Yu et al., 2009). Clinical practice of CQ was banned because of problems regarding the development of subacute myelo-optico-neuropathy (SMON) (Tateishi, 2000). Recently the interest in CQ and CQ-like compounds reemerges as a possible drug for Alzheimer's disease (Cherny et al., 2001; Bush and Tanzi, 2008; Grossi et al., 2009). Administration of CQ seems to be able to solubilize β-amyloid plaques by chelating zinc and cupper in transgenic models of Alzheimer's disease. Clinical trials have been performed to test for possible beneficial effects of CQ in Alzheimer's disease patients (Ritchie et al., 2003).

CQ has a relatively weak affinity for zinc (K_d, approximately 1×10^{-7} M) unlike tetrakis-(2-pyridylmethyl) ethylendediamine (TPEN), ($K_d=2.6\times10^{-16}$ M) and is estimated to interact with loosely bound zinc, that is histochemically reactive zinc (Cherny et al., 2001). To evaluate the role of histochemically reactive zinc in memory formation, in the present study, hippocampal LTP induction and cognitive

*Corresponding author. Tel: +81-54-264-5700; fax: +81-54-264-5705.
E-mail address: takedaa@u-shizuoka-ken.ac.jp (A. Takeda).

Abbreviations: ACSF, artificial cerebrospinal fluid; CQ, clioquinol or 5-chloro-7-iodo-8-hydroxyquinoline; fEPSP, field excitatory postsynaptic potentials; HFS, high frequency stimulation; LTP, long-term potentiation; PS, population spike; SMON, subacute myelo-optico-neuropathy.

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behavior were examined in young rats after i.p. injection of CQ (30 mg/kg).

EXPERIMENTAL PROCEDURES

Animals and chemicals

Male Wistar rats (6-weeks old) were purchased from Japan SLC (Hamamatsu, Japan). There were housed under the standard laboratory conditions (23±1 °C, 55±5% humidity) and had access to tap water and food *ad libitum*. Several days later, rats were used for experiments. All experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka.

CQ was dissolved in 20% dimethyl sulfoxide (DMSO) in olive oil. ZnAF-2, a membrane-impermeable zinc indicator, was kindly supplied from Sekisui Medical Co., LTD (Tokai, Japan), dissolved in DMSO, and then diluted to artificial cerebrospinal fluid (ACSF) containing 119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 26.2 mM $NaHCO_3$, and 11 mM D-glucose (pH 7.3).

Timm's sulfide-silver staining

Rats were deeply anesthetized with chloral hydrate (n=12) and then perfused transcardially with 0.1% Na_2S in phosphate buffer (pH 7.4). The brains were excised and immersed in 4% (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) for 24 h and then in 10–30% sucrose for 72 h. Coronal 30 μm sections were prepared in a cryostat at −20 °C. Timm's staining was performed according to the procedure described previously (Danscher, 1981). The intensity of Timm's stain was measured by using Multi Gauge V3.1 (Fuji Photo Film, Tokyo, Japan).

Hippocampal slice preparation and zinc imaging

Rats were anesthetized with ether and decapitated. The brain was quickly removed and immersed in ice-cold ACSF. Transverse hippocampal slices (400 μm) were prepared using a vibratome ZERO-1 (Dosaka Kyoto, Japan) in an ice-cold ACSF. Slices were then maintained in a holding chamber at room temperature for at least 1 h. All solutions used in the experiments were continuously bubbled with 95% O_2 and 5% CO_2.

For extracellular zinc imaging, the hippocampal slices were transferred to a recording chamber filled with 10 μM ZnAF-2 in ACSF (n=7). The fluorescence of ZnAF-2 (excitation, 488 nm; monitoring, 505–530 nm) was measured in the hippocampus by using a confocal laser-scanning microscopic system LSM 510 (Carl Zeiss), equipped with the inverted microscope (Axiovert 200M, Carl Zeiss). Region of interest was set in the molecular layer of the dentate gyrus, the stratum lucidum, and the stratum radiatum of the CA1.

Dentate gyrus LTP

Dentate gyrus LTP was recorded under anesthesia as reported previously (Fukazawa et al., 2003). Male Rats were anesthetized with chloral hydrate (400 mg/kg) and placed in a stereotaxic apparatus (n=5–6). A bipolar stimulating electrode and a monopolar recording electrode made of tungsten wire were positioned stereotaxically so as to selectively stimulate the perforant pathway while recording in the dentate gyrus. The electrode stimulating the perforant pathway fibers was implanted 8.0 mm posterior, 4.5 mm lateral, 3.0–3.5 mm inferior to the bregma. A recording electrode was implanted ipsilaterally 4.0 mm posterior, 2.5 mm lateral and 3.0–3.5 mm inferior to the bregma. All the stimuli were biphasic square wave pulses (200 μs width) and their intensities were set at the current that evoked 40% of the maximum population spike (PS) amplitude. Test stimuli (0.05 Hz) were delivered at 20 s intervals to monitor field excitatory postsynaptic potentials (fEPSP).

At the beginning of the experiments, input/output curves were generated by systematic variation of the stimulus current (0.1–1.0 mA) to evaluate synaptic potency. After stable baseline recording for at least 30 min, LTP was induced by delivery of high-frequency stimulation (HFS), (10 trains of 20 pulses at 200 Hz separated by 1 s).

Object recognition memory

Rats were placed for 10 min into an open field, which was a 70×60 cm^2 arena surrounded by 70 cm high walls, made of a black-colored plastic, once a day for 2 days (n=10–14). Twenty-four hours after open field exploration, rats were trained and tested in a novel object recognition task as reported previously (Pietá Dias et al., 2007). Training in the object recognition task took place in the same area used for the open field exploration. The open field exploration was thus used as a context habituation trial for the recognition memory task. The object recognition test requires that the rats recall which of two earthenware objects they had been previously familiarized with. Twenty-four hours after arena exploration, training was conducted by placing individual rats into the field, in which two identical objects (objects A1 and A2; sake bottle) were positioned in two adjacent corners, 15 cm from the walls. Rats were left to explore the objects for 5 min. Rats were not used for the test when the total of the object exploration time was less than 20 s. In a short-term memory test given 1 h after training, the rats explored the open field for 3 min in the presence of one familiar (A) and one novel (B; cup) object. All objects presented similar textures, colors and sizes, but distinctive shapes. A recognition index calculated for each rat was expressed by the ratio $T_B/(T_A+T_B)$ [T_A = time spent to explore the familiar object A; T_B = time spent to explore the novel object B]. Between trials the objects were washed with 70% ethanol solution. In a long-term memory test given 24 h after training, the rats were subjected to the test in the same manner. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on the object was not considered as exploration.

Behavior and locomotor activity of rats were also assessed in the open field when they were subjected to training of the object recognition task. Behavior of each rat in the arena was recorded with a video camera and locomotor activity, standing behavior and grooming behavior were measured for 5 min.

Statistical analysis

Field EPSP amplitudes (test frequency: 0.05 Hz) were averaged over 120-s intervals and expressed as percentages of the mean fEPSP amplitude measured during the 30-min baseline period perfused with ACSF prior to LTP induction. Grouped data are expressed as the mean±SEM. For statistical analysis, the Dunnett's test that was used to make multiple comparisons with the control (vehicle) group, two-way ANOVA, and Student's t-test were also used.

RESULTS

Transient reduction of histochemically reactive zinc and its extracellular levels

Mice injected i.p. with CQ (30 mg/kg) shows a dramatic reduction in histochemically reactive zinc in the brain, testis and pancreas (Nitzan et al., 2003). Repeated oral treatment with CQ (30 mg/kg/day) in Tg2576 mice results in a reduction of cortical deposition of amyloid with an improvement or stability in the general health and weight param-