Neuroscience 139 (2006) 463–473

LEAD INHIBITED *N*-METHYL-D-ASPARTATE RECEPTOR-INDEPENDENT LONG-TERM POTENTIATION INVOLVED RYANODINE-SENSITIVE CALCIUM STORES IN RAT HIPPOCAMPAL AREA CA1

X. M. LI, Y. GU, J. Q. SHE, D. M. ZHU, Z. D. NIU, M. WANG, J. T. CHEN, L. G. SUN AND D.-Y. RUAN*

School of Life Sciences and Institute of Polar Environment, University of Science and Technology of China, Hefei, Anhui 230027, PR China

Abstract—Lead exposure is known to be associated with cognitive dysfunction in children. Impairment of the induction of long-term potentiation (LTP) has been reported in area CA1 of rat hippocampus following lead exposure *in vivo* and *in vitro*. The present study was carried out to investigate whether the alterations of *N*-methyl-D-aspartate (NMDA) receptor-independent LTP following lead exposure involve internal calcium stores in hippocampus CA1 synapses. Monosynaptic field excitatory postsynaptic potentials in hippocampal slice area CA1 were recorded using the whole-cell patch-clamp upon acute lead treatment, and these studies were coupled with calcium imaging experiments to observe internal calcium changes in cultured hippocampal neurons. Inhibiting calcium release by ryanodine significantly reduced NMDA receptor-independent LTP, and depletion of internal calcium stores with thapsigargin blocked this form of LTP. Caffeine, an agonist of ryanodine receptors, enhanced this form of LTP. However, caffeine-enhanced NMDA receptor-independent LTP was depressed after bath application of lead. Moreover, lead further decreased ryanodine- and thapsigargin-reduced NMDA receptor-independent LTP. Calcium imaging also confirmed that lead had an effect on internal calcium release and uptake. Taken together, these results demonstrated that lead inhibited NMDA receptor-independent LTP by action on calcium release and uptake by ryanodine-sensitive stores in rat hippocampal area CA1.

Key words: NMDA receptor-independent LTP, ryanodine receptors, internal calcium stores, lead.

Lead (Pb) was known to be a potent neurotoxic agent in our environment. Lead exposure causes a wide variety of detrimental effects on CNS. Prominent neurotoxic effects of lead exposure are the impairments in learning and memory (Needleman, 1992). Hippocampal long-term potentiation (LTP) is a useful animal model for electrophysiologic study of synaptic plasticity, and is believed to be a component of learning and memory (Bliss and Collingridge, 1993). Two distinct forms of LTP including *N*-methyl-D-aspartate (NMDA) receptor-dependent and NMDA receptor-independent have been found in the vertebrate CNS (Collingridge et al., 1983; Harris and Cotman, 1986). Previous studies displayed that lead exposure inhibited LTP through various pathways, such as NMDA receptor, protein kinase C and others (Hussain et al., 2000; Nihei et al., 2000). Although some studies suggest lead impairs LTP by an action not on NMDA receptors in hippocampus (Hori et al., 1993), the mechanism remains unclear.

Recent studies have established an important role for internal calcium stores in the induction of NMDA receptor-independent LTP (Lauri et al., 2003). Two types of calcium stores including ryanodine receptors (RyRs)-sensitive and inositol 1,4,5-trisphosphate receptors (IP_3Rs)-mediated, are located in endoplasmic reticulum (ER) and constitute a large and important calcium source for the induction of NMDA receptor-independent LTP (Berridge, 1998; Mattson et al., 2000). Blockage of ER calcium release by ryanodine has been shown to inhibit this form of LTP in CA3 (Lauri et al., 2003). The highest level of expression of the brain-type RyR 3 (RyR_3) is in CA1 hippocampal pyramidal neurons (Furuichi et al., 1994). The results indicated that ryanodine-sensitive calcium stores maybe contributed to synaptic plasticity in hippocampal CA1 pyramidal neurons. Modulation of intracellular calcium concentration-involved introduction and expression of LTP was crucial to the neurotoxicity of lead (Simons, 1993). There was considerable evidence that lead inhibited LTP by an action not on NMDA receptors (Hori et al., 1993), since blockade of LTP by lead did not depend on an inhibitory effect of lead on the NMDA receptors. We, thus, hypothesized that lead inhibited NMDA receptor-independent LTP via internal calcium stores.

The present study was initiated to understand how ryanodine-sensitive calcium stores contributed to the effect of lead on NMDA receptor-independent LTP. (1) We confirmed that ryanodine-sensitive calcium stores were involved in NMDA receptor-independent LTP in area CA1 of hippocampus slice. (2) Lead reduced NMDA receptor-independent LTP in hippocampal CA1 involved the release and uptake of ryanodine-sensitive calcium stores.

*Correspondence to: D.-Y. Ruan, Department of Neurobiology and Biophysics, School of Life Sciences, University of Science and Technology of China, Hefei, Anhui 230027, PR China. Tel: +86-551-3606374; fax: +86-551-3601443.
E-mail address: ruandy@ustc.edu.cn (D.-Y. Ruan).

Abbreviations: ACSF, artificial cerebrospinal fluid; D-AP5, D-(−)-2-amino-5-phosphomopentanoic acid; EPSCs, excitatory postsynaptic currents; EPSPs, excitatory postsynaptic potentials; ER, endoplasmic reticulum; IP3, inositol 1,4,5-trisphosphate; LTP, long-term potentiation; NMDA, *N*-methyl-D-aspartate; RyRs, ryanodine receptors.

0306-4522/06$30.00+0.00 © 2005 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2005.12.033

EXPERIMENTAL PROCEDURES

Cell culture

Low-density cultures of dissociated embryonic rat hippocampal neurons were prepared as described (Bi and Poo, 1998) with some modifications. Hippocampi were removed from E17 to E20 embryonic rats and treated with trypsin for 12–15 min at 37 °C, followed by gentle trituration. The dissociated cells were plated at densities of 25,000–60,000 cells/ml on poly-L-lysine-coated glass coverslips in 35 mm Petri dishes. The plating medium was Dulbecco's minimum essential medium (DMEM, Gibco BRL, Rockville, MD, USA) supplemented with 10% fetal calf serum (Gibco), 10% Ham's F12 with glutamine (Gibco). Twenty-four hours after plating, the culture medium was changed to a maintenance medium containing neurobasal media and 5% B-27 supplement (Gibco). Cultured neurons were used for confocal observer after one week's culturing.

Slice preparation

Hippocampal slices were prepared as described previously (Kato et al., 1993). Sprague–Dawley rats (15–18 days old) were anesthetized with sodium pentobarbital. After decapitation, hippocampal formation was dissected rapidly and placed in ice-cold oxygenated solution containing (in mM) 119 NaCl, 26.2 $NaHCO_3$, 11 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, and 1.3 $MgSO_4$, pH 7.2. Transverse slices (300 μm thick) were cut with a vibratome (DTK-1000; Dosaka, Kyoto, Japan) and maintained in an incubation chamber for at least 1 h at room temperature before recording. During the experiments, individual slices were transferred to a submersion recording chamber, where they were continuously perfused with the above extracellular solution (2.0–3.0 ml/min) at 28 °C–29 °C. Slices were viewed with an Olympus microscope (DX50WI), using infrared video microscopy (model E600-FN, Nikon Japan) and differential interference contrast optics.

Electrophysiology

Whole cell patch-clamp recordings were performed in CA1 pyramidal neurons according to standard techniques, using a patch-clamp amplifier EPC9 (HEKA, Germany). Patch pipettes were pulled from 1.2 mm outside diameter borosilicate glass. The pipettes (3–5 MΩ resistance) were filled with a solution containing (in mM): 125 K-glucose, 15 KCl, 4 $MgCl_2 \cdot 6H_2O$, 3 Na_2ATP, 0.3 Na GTP, 0.2 EGTA, 5 QX-314 and 10 HEPES with pH adjusted to 7.2 using KOH and 290–300 mOsm. The resting membrane potential was approximately −65 mV. The membrane potential was maintained at a level of about −75 mV by injection of a constant current (<100 pA). The membrane potential for most neurons was stable throughout the recording. Input resistance was 100–200 MΩ and was checked repeatedly in some neurons by measuring the voltage response to a step-hyperpolarizing current pulse (30 pA, 150 ms) throughout the experiments. In all cases for which input resistances were monitored, we found no change in the input resistance during control recording and after LTP induction (n=6). Glass pipettes (tip diameter 2–3 MΩ) filled with artificial cerebrospinal fluid (ACSF) were used as extracellular-stimulating electrodes and were connected to stimulators (S88K, Grass Instruments) and isolators (SIU7, Grass Instruments). Monosynaptic excitatory postsynaptic potentials (EPSPs) and excitatory postsynaptic currents (EPSCs) were elicited with the latency between stimulus and EPSP onset less than 3 ms. In a typical experiment, the stimulating electrodes were placed in the s. radiatum at distances of about 150 μm, distal from the cell body layer. EPSPs and EPSCs were evoked every 20 s. The initial slopes of EPSPs and peak amplitudes of EPSCs were measured for EPSP and EPSC, respectively. Picrotoxin 100 μM was included in the perfusate throughout recordings. Lead acetate was freshly dissolved to 0.5–1 mM with deionized water, diluted to end concentrations with ACSF just before the experiments. Although the concentration of 20 μM exceeds that of lead solubility in our bath, we used this concentration in order to assure maximal lead solubility under the conditions of our experiments (Hori et al., 1993; Hussain et al., 2000). In some experiments we applied 10 mM caffeine in the bath solution to activate RyRs. Ryanodine (10 μM), and thapsigargin (1–2 μM) were included in the pipette internal solution throughout recording in some experiments. Tetanic stimuli were always delivered in the presence of the competitive NMDA receptor antagonist D-(−)-2-amino-5-phosphonopentanoic acid (D-AP5, 50 μM) to ensure that only NMDA receptor-independent LTP was investigated. All reagents were purchased from Sigma (St. Louis, MO, USA).

Calcium imaging

Primary culture neurons were washed with Ringer's saline containing (in mM): 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 12 glucose, and buffered to pH 7.4. The saline was continuously bubbled with carbogen (95% O_2, 5% CO_2). Cells were loaded with 10 μM fluo-4-AM (Molecular Probes, Eugene, OR, USA) with pluronic F-127 [Molecular Probes, 0.004% (w/v) final] in a ACSF (in mM) (125 NaCl, 3 KCl, 10 glucose, 26 $NaHCO_3$, 1.1 NaH_2PO_4, 2 $CaCl_2$, 1 $MgSO_4$; pH adjusted to 7.4) at room temperature for 20–25 min. Endogenous esterases converted nonfluorescent fluo-4-AM into fluorescent fluo-4. For acute lead exposure or ryanodine treatment, cells were pretreated with 10 μM lead or 10 μM ryanodine for 90 min at least. Cultures were imaged with a Biorad MC1024 confocal microscope (Bacskai et al., 1995). Cells were continuously perfused with Ringer's bath solution flowing at 5 ml/min, and caffeine or ryanodine was microperfused onto an individual cell through a quartz perfusion head. Epifluorescent excitation for fluo-4 was at the 488 nm line of an argon-ion laser (Coherent, Santa Clara, CA, USA), and emission was collected at 510 nm. For measurements of calcium-dependent changes in the fluorescence a sequence of images was sampled in one focal plane. Images of calcium measurements were taken with each 30 s. Camera gain was adjusted to give baseline maximal fluorescence levels of 40–100 (arbitrary units) of a maximal eight-bit signal output of 256. Fluorescence measurements after caffeine for each cell (F) were normalized to the average fluorescence intensity. Cell fluorescence during the 6 min baseline period was F0. Region of indexes (ROIs) were defined in the first image, and the normalized fluorescence changes (F−F0)/F0, that is ΔF/F0, were measured throughout the image sequence. All settings of the lasers, filters, and microscope and the complete data acquisition were controlled by Lasersharp software (Biorad).

Data collection and analysis

Data were recorded at least 20 min after pipette seal on the soma, and filtered at 2 kHz, digitized at 10 kHz, and stored by ITC-18 card and Pulse 8.53 software (HEKA Instruments), and analyzed offline with Igor Pro 4.0 software (Wavemetrics). For this experiment, an initial strength of stimulus with amplitude in the range of 6–10 mV was chosen to evoke the individual EPSPs, followed by stimuli at other strengths (10 repetitions at 0.0033 Hz for each measurement of EPSP amplitude). EPSCs were 40% of maximal evoked amplitudes. EPSCs were evoked at a stimulation rate of 0.033 Hz. LTP was induced by four 200 Hz stimulus trains (0.5 s duration) delivered at 5 s intervals. Stimulus intensity during tetanization was set to twice the test stimulus intensity. Statistical analysis of the data was provided as means±S.E.M., and statistical significance was established by a one-way ANOVA, followed by post hoc tests.