BIOMEDICAL AND ENVIRONMENTAL SCIENCES 15, 330-340 (2002)

Lead Can Inhibit NMDA-, K^+-, QA/KA-Induced Increases in Intracellular Free Ca^{2+} in Cultured Rat Hippocampal Neurons[1]

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Objective To examine the effects of Pb^{2+} on N-methyl-D-aspartate (NMDA)-, K^+- and quisqualate(QA)/kainite(KA)-induced increases in intracellular free calcium concentration ($[Ca^{2+}]_i$) in cultured fetal rat hippocampal neurons in order to explain the cognitive and learning deficits produced by this heavy metal. **Methods** Laser scanning confocal microscopy was used. **Results** The results clearly demonstrated that adding Pb^{2+} before or after NMDA/glycine stimulation selectively inhibited the stimulated increases in $[Ca^{2+}]_i$ in a concentration-dependent manner. In contrast, Pb^{2+} treatment did not markedly affect increases in $[Ca^{2+}]_i$ induced by an admixture of QA and KA. The minimal inhibitory effect of Pb^{2+} occurred at 1 μmol/L, and more than seventy percent abolition of the NMDA-stimulated increase in $[Ca^{2+}]_i$ was observed at 100 μmol/L Pb^{2+}. Evaluation of Pb^{2+}-induced increase in $[Ca^{2+}]_i$ response to elevating extracellular concentrations of NMDA, glycine or calcium revealed that Pb^{2+} was a noncompetitive antagonist of both NMDA and glycine, and a competitive antagonist of Ca^{2+} at NMDA receptor channels. In addition, Pb^{2+} inhibited depolarization-evoked increases in $[Ca^{2+}]_i$ mediated by K^+ stimulation (30 μmol/L), indicating that Pb^{2+} also depressed the voltage-dependent calcium channels. Also, the results showed that Pb^{2+} appeared to be able to elevate the resting levels of $[Ca^{2+}]_i$ in cultured neurons, implying a reason for Pb^{2+}-enhanced spontaneous release of several neurotransmitters reported in several previous studies. **Conclusion** Lead can inhibit NMDA-, K^+-, QA/KA-induced increases in intracellular $[Ca^{2+}]_i$ in cultured hippocampal neurons.

Key words: Lead poisoning; Glutamate receptor; Rat hippocampal neuron; Calcium; Learning deficit; Laser scanning confocal microscopy

INTRODUCTION

Lead (Pb^{2+}) poisoning remains a significant public health problem for children because of its neurotoxicity, which can result in a significant deficit in cognitive development[1-3]. Although considerable research efforts have been made, the mechanisms underlying these Pb^{2+}-induced cognitive impairments have not been defined[2, 4-10].

Central neurons express N-methyl-D-aspartate (NMDA) and voltage-activated calcium channels. Activation of these channels and resultant calcium influx have been shown to be essential for the induction of long-term potentiation (LTP), a potential neurobiological substrate of learning and memory processes[11]. In addition, it has been recently shown that

[1] This work was supported by the Chinese Academy of Preventive Medicine Fund.

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0895-3988/2002
CN 11-2816

certain subpopulations of central neurons express quisqualate(QA)/kainate(KA) receptor gatting channels with direct Ca^{2+} permeability[12], and that this receptor channel complex is also necessary for the induction of LTP[11]. In view of the function of these calcium channels in learning and memory processes and the fact that to date it is not yet clear whether Pb^{2+}-induced cognitive deficits involve interference with the functions of the above calcium channels, the effects of Pb^{2+} on calcium channel function were investigated by imaging intracellular free calcium concentration ($[Ca^{2+}]_i$) in primary hippocampal neurons during NMDA, KA/QA and potassium stimulation.

MATERIALS AND METHODS

Materials and Chemical Reagents

Fluo-3/AM, lead acetate (PbAc, 99.8% purity), NMDA, DL-2-amino-5-phosphonovaleric acid (AP-5), KA, QA, pluronic F-127 and HEPES were purchased from Sigma Chemical Co., St. Louis, and Mo. Cell culture materials were obtained from Gibco, Grand Island, NY. All other chemicals were commercial products of the highest available grade of quality. Adherent cell analysis and sorting system (ACAS)/Ultima 212 laser scanning confocal microscope was obtained from Meridian Instrument, Okemos, while MI. Plastic petri dishes (35 mm) were purchased from Corning Glassware, Corning, NY.

HEPES-buffered Hanks (HBH) solution used in cell loading had the following compositions (mmol/L): HEPES 20, NaCl 137, $CaCl_2$ 2.2, KCl 5.0, KH_2PO_4 0.4, Na_2HPO_4 0.6, $NaHCO_3$ 3.0 and glucose 5.6. The pH of the solution was adjusted to 7.4 with NaOH. The recording buffer (RB) used in monitoring Fluo-3 fluorescence consisted of (mmol/L): NaCl 118.5, KCl 4.8, $CaCl_2$ 1.3, glucose 11.1, ascorbic acid 1.1 and HEPES 17, and the pH was adjusted to 7.0 with Tris base. Stock solutions of PbAc, dissolved in 0.15 mol/L NaCl, and KA, QA or AP-5 or Mg^{2+}, dissolved in RB, were fast applied by micropipette to 1 mL RB in test dish. The addition of each of these reagents was restricted to 5 μL volume so that changes of total solution volume in the dish could be ignored. Glycine (10 μmol/L) was added as a coagonist[13,14] to all NMDA exposures (except as indicated). KA and QA were employed as an admixture.

TISSUE CULTURE

Dissociated hippocampal cell suspensions were prepared from 1-day-old Wistar rat pups and plated on 35 mm plastic petri dishes pretreated with poly-L-lysine at $1\text{-}2\times10^5$ cells/cm^2, as described previously[5]. The initial plating medium was a 2:1 mixture of Dulbecco's modified Eagle's medium: Ham's nutrient F12 medium supplemented with 10% heat-inactivated horse serum, 10% heat-inactivated fetal bovine serum and 1% antibiotic solution (100 units/mL penicillin and 100 μg/mL streptomycin). Cultures were maintained at 36.5℃ in 5% CO_2. One day after plating, the culture medium was switched into a maintenance medium which was identical to the plating medium but lacking fetal bovine serum. Subsequent media replacement occurred twice a week by replacing 50% medium with fresh maintaining medium. After 3-5 d *in vitro*, non-neuronal cell division was halted by exposure to 10 μmol/L cytosine arabinoside for 1-3 d. Cultures were used for experiments after 9-14 d *in vitro*.

Confocal Measurement of $[Ca^{2+}]_i$

Changes in $[Ca^{2+}]_i$ were estimated using the fluorescent membrane permeable probe fluo-3/AM. The fluorescence intensity of fluo-3 increases in presence of Ca^{2+}. Prior to fluorescence measurement, the cultures were first loaded with fluo-3 by incubating them at 36.5℃ for 30-60 min in the dark in HBH containing 4 μmol/L fluo-3/AM, 0.125% (w/v) pluoronic acid (as solubilizing agent), and 1.5% (v/v) dimethylsulfoxide (DMSO). The cultures were then washed twice with the RB, and 1 mL of fresh RB was added.

The test dish containing preloded cells was set on the motorized stage of an ACAS/ Ultima 212 laser scanning confocal microscope equipped with an argon/krypton laser to excite the dye at 488 nm. Fluorescence emission above 510 nm was measured after passing through a long pass filter in front of the photomultiplier tube. The confocal system was employed in the slow image scan mode. In this mode, a sequence of video images, constructed from 200×200 pixels for single selected cells from different visualizable fields, was collected every 20-30 s. The initial 4-6 scans (within about 100 s) were performed to determine calcium baseline; later scans were made to show the time course of $[Ca^{2+}]_i$ changes in single cell level in response to agent applications. The acquired fluorescence data were stored in and analyzed by the computer designed for the confocal microscope. $[Ca^{2+}]_i$ in single cells was expressed as fluorescence intensity or normalized data, which were the ratio of each scan data to the first scan data.

RESULTS

Inhibitory Effects of Pb^{2+} *on NMDA-induced Increases in* $[Ca^{2+}]_i$

As shown in Table 1, addition of 50 μmol/L NMDA produced a pronounced increase in $[Ca^{2+}]_i$. This increase could be inhibited by AP-5, a competitive NMDA receptor antagonist or by Mg^{2+}, a non-competitive NMDA receptor antagonist. One hundred μmol/L AP-5 added simultaneously with 50 μmol/L NMDA or 1.0 mmol/L Mg^{2+} added prior to 50 μmol/L NMDA inhibited the expected rise in $\Delta[Ca^{2+}]_i$ by about 56% and 45%, respectively. Also, addition of Mg^{2+} after agonist stimulation reversed the stimulated increases in $[Ca^{2+}]_i$. As exemplified in Fig. 1, the addition of 1.0 mmol/L Mg^{2+} greatly accelerated the return of NMDA-induced increases in $[Ca^{2+}]_i$. This accelerated decrement was again observed after the addition of 2.0 mmol/L Mg^{2+}. The above results suggested that NMDA's effects were mediated through its own receptors.

TABLE 1

Effect of Mg^{2+} and AP-5 on NMDA-Induced Increases in $[Ca^{2+}]_i$

Condition	$\Delta[Ca^{2+}]_i$
NMDA	507.5±82.9
AP-5/NMDA mixture	225.8±139.8
Mg^{2+}+NMDA	277.3±42.4

Note. NMDA antagonists, AP5 (100 μmol/L) or Mg^{2+} (1.0 mmol/L), were added either simultaneously with or 80 sec before 50 μmol/L NMDA. $[Ca^{2+}]_i$ was scanned continuously and $\Delta[Ca^{2+}]_i$ represents the net increase of fluorescence intensity after subtracting pre-NMDA levels; Values represent $\bar{x} \pm s$ determined from three experiments (8-20 cells in each condition).

Addition of 25 μmol/L Pb^{2+} prior to or after 50 μmol/L NMDA exposure prevented (Fig. 2,

labelled as 4-7) or reversed (Fig. 3) the increases in $[Ca^{2+}]_i$ induced by the agonist. The effects of Pb^{2+} resembled the blocking actions of AP-5 or Mg^{2+} on the NMDA receptor channels. To rule out a false effect that a mechanical stimulation by adding a solution might cause on the $[Ca^{2+}]_i$ we applied the RB as control about 80 sec before or 90 sec after NMDA stimulation. No decrements in the stimulatory response were observed, as demonstrated by the images labelled as 0-3 in Fig. 2 or by the unchanged partial curve at the middle arrow in Fig. 3. These findings demonstrated that Pb^{2+} could inhibit the NMDA-induced increases in $[Ca^{2+}]_i$.

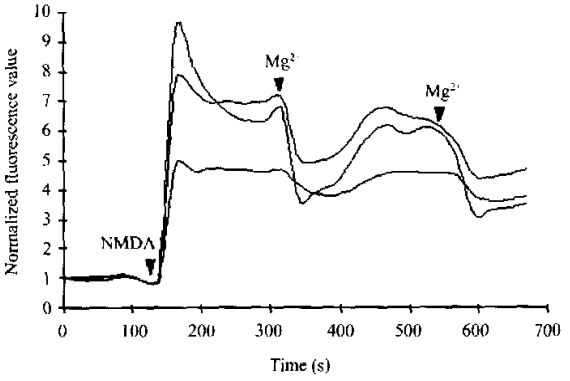

FIG. 1. Reversing effect of Mg^{2+} on the NMDA-activated increases in $[Ca^{2+}]_i$. Three cells from one dish were scanned successively and data were expressed as normalized fluorescence values. Resting $[Ca^{2+}]_i$ was monitored for about 120 s followed by 50 μmol/L NMDA stimulation (first arrow). After 180 s 1.0 mmol/L Mg^{2+} was added into the dish (second arrow) causing a small reversal of the NMDA-induced increases in $[Ca^{2+}]_i$. About 260 s later, 2.0 mmol/L Mg^{2+} was added and a further decline in fluorescence increases occurred (third arrow).

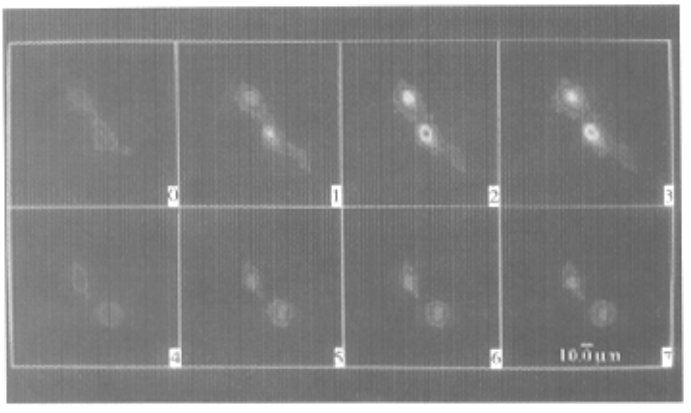

FIG. 2. Inhibitory effect of Pb^{2+} on the NMDA-induced increases in $[Ca^{2+}]_i$. Two series of selected sequential images of two neurons exposed either to RB (control, upper series) or 25 μmol/L Pb^{2+} (low series) at approximately 80 s before 50 μmol/L NMDA addition were obtained. Corresponding images labeled by 0 and 4, 1 and 5, 2 and 6, 3 and 7 denoted images taken respectively just before or at 20, 40, 60 s after NMDA stimulation.

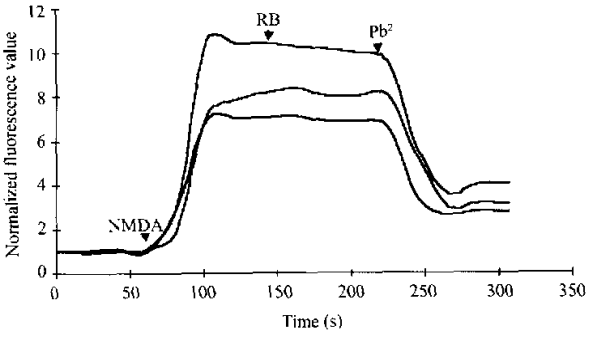

FIG. 3. Reversing effect of Pb^{2+} on the NMDA-activated increases in $[Ca^{2+}]_i$ in three cells from the same dish. Cells were scanned successively and data were expressed as normalized fluorescence values. Resting $[Ca^{2+}]_i$ was monitored for 60 s followed by the addition of 50 μmol/L NMDA. After approximately 100 s the RB solution was added into the dish to investigate a possible mechanical effects on $[Ca^{2+}]_i$. About 80 s later, 25 μmol/L Pb^{2+} was added, resulting in a rapid decrement in the NMDA- stimulatory response.

Dose-effect Relationship for the Inhibitory Effects of Pb^{2+} *on NMDA-induced Increases in* $[Ca^{2+}]_i$

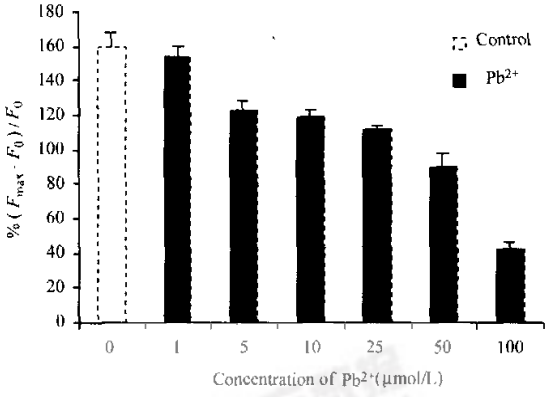

FIG. 4. Concentration-related effect of Pb^{2+}-induced depression in $[Ca^{2+}]_i$. Resting $[Ca^{2+}]_i$ was monitored for about 80 s followed by the addition of 0.15mol/L NaCl (control) or Pb^{2+} (1, 5, 10, 25, 50, 100 μmol/L in 0.15 mol/L NaCl). After 80 s, 50 μmol/L NMDA was added and fluorescence monitored for 120 s. Data were expressed as a change in fluorescence over baseline fluorescence % $(F_{max}-F_0)/F_0$ for each cell, where F_{max} refereed to the NMDA-stimulated maximal increases in fluorescence intensity and F_0 represented the pre-NMDA fluorescence levels. Dividing the F values by an F_0 provides correction for differences in the length of light paths, and within certain limits the fluo-3 concentrations. Value of each column represents $\bar{x}\pm s$ of 8-12 single cells. Correlation analysis showed a significant concentration-dependence inhibition by Pb^{2+} on NMDA responses (t=3.18, P<0.05)

When increasing doses of Pb^{2+} were added to the cultures prior to 50 μmol/L NMDA stimulation, the peak amplitudes of stimulated fluorescence intensity were depressed by Pb^{2+} in a concentration-dependent manner. This depression was detected at concentrations as low as 1.0 μmol/L Pb^{2+} and more than seventy percent of abolition of the NMDA stimulation was observed at 100 μmol/L Pb^{2+} as compared to the control (Fig. 4). Moreover, the sequential addition of increasing concentrations of Pb^{2+} resulted in stepwise reversal of NMDA-induced increases in $[Ca^{2+}]_i$ albeit the amplitude of the effects was small (Fig. 5). The lowest effective dose of Pb^{2+} here was also 1.0 μmol/L.

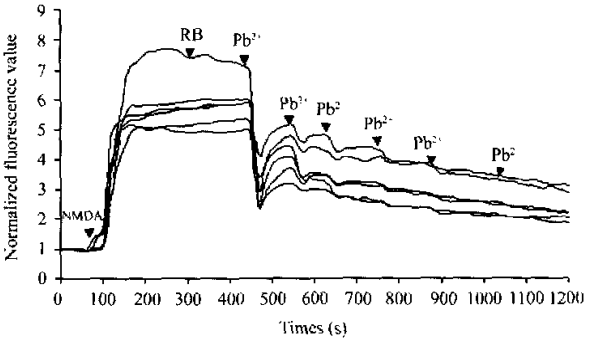

Fig. 5. Time plot showing concentration dependence of Pb^{2+}-caused reversal of NMDA-induced increases in $[Ca^{2+}]_i$. After monitoring resting $[Ca^{2+}]_i$ for about 90 s, 50 μmol/L NMDA was added, resulting in a rapid and marked increase in $[Ca^{2+}]_i$. After 200 s, RB (control) and 6 increasing doses of Pb^{2+} (1, 5, 10, 25, 50, 100 μmol/L in 0.15 mol/L NaCl) were sequentially added at about 100 s interval (as indicated by arrows), resulting in a stepwise reversal of the fluorescence intensity following each addition.

Mechanisms of Action of Pb^{2+} *at the NMDA Receptor in Cultured Neurons*

To elucidate the mode of action of Pb^{2+}, the dependence of Pb^{2+}-caused inhibition on the concentrations of NMDA, glycine and Ca^{2+} was investigated. As shown in Fig. 6, Pb^{2+} appeared to be a noncompetitive antagonist of glycine or NMDA in particular, because raising the concentration of NMDA (from 50 μmol/L to 100 μmol/L) or glycine (from 10 μmol/L to 100 μmol/L) after eliciting the blocking action of Pb^{2+} failed to reverse the Pb^{2+} action. In contrast, when the extracellular concentration of Ca^{2+} was raised from 2.2 mmol/L to 5.0 mmol/L after eliciting Pb^{2+}-induced reversal, Ca^{2+} could antagonize the action of Pb^{2+}, suggesting that Ca^{2+} could compete with Pb^{2+} at NMDA receptor channels, or in other words, Pb^{2+} could antagonize the action of Ca^{2+} at the channels.

Inhibition of Depolarization-triggered Increases in $[Ca^{2+}]_i$ *by* Pb^{2+}

Rapid and marked increases in $[Ca^{2+}]_i$ were resulted from high potassium application (Figs. 7 and 8). As shown in Fig. 7, the normalized fluorescence intensity values recorded from seven cells after 30 mmol/L K^+ increased by 6-10 fold relative to the prior stimulation levels with enhanced fluorescence decaying back to resting levels slowly. When 50 μmol/L and 100 μmol/L Pb^{2+} was added sequentially to the cells in the decaying processes, separate and transient accelerated decrements in fluorescence were produced. In addition, when 25 μmol/L Pb^{2+} was added to the cells about 80 s before 30 mmol/L K^+ stimulation, the

amplitude of high K^+-evoked increases in $[Ca^{2+}]_i$ was decreased markedly (Fig. 8). These results suggested that Pb^{2+} could block voltage-dependent calcium channels in the cultured neurons.

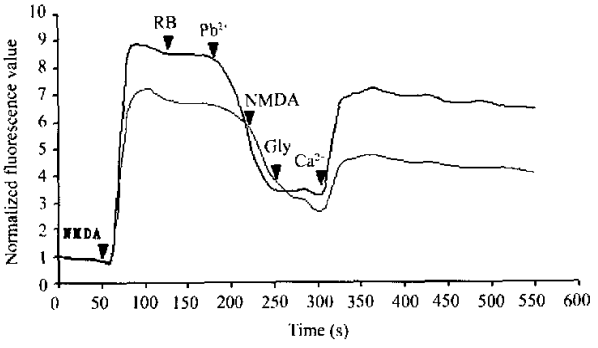

FIG. 6. Effects elevating extracellular concentrations of NMDA, glycine and calcium on Pb^{2+}-induced reversal of NMDA-triggered increases in $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was scanned successively and 50 μmol/L NMDA was added (at the time point labelled by the first arrow), resulting in a rapid increase in $[Ca^{2+}]_i$. About 60 s later, RB, 25 μmol/L Pb^{2+}, 100 μmol/L NMDA, 100 μmol/L glycine and 5 mmol/L Ca^{2+} were added sequentially (at the time points indicated by other 5 arrows).

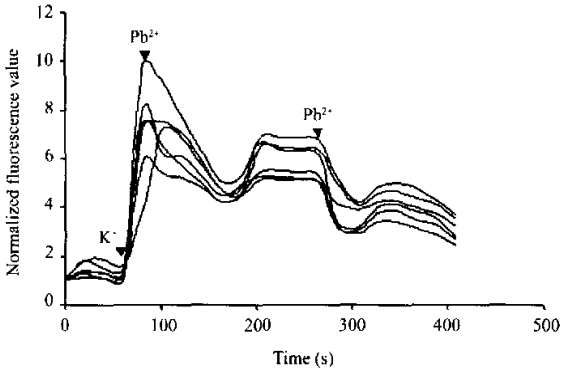

FIG. 7. Reversing effect of Pb^{2+} on K^+-triggered increases in $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was monitored successively and the data were expressed as normalized fluorescence intensity. After establishing calcium baselines, 30 mmol/L K^+ was applied to the cells resulting in rapid increases in $[Ca^{2+}]_i$. 50 μmol/L Pb^{2+} was added immediately after eliciting the maximal effects of K^+ and 100 μmol/L of this cation was added about 180 s later. Each addition of Pb^{2+} resulted in a transient reversal of the K^+-triggered increases in $[Ca^{2+}]_i$.

Effect of Pb^{2+} *on KA/QA-triggered Increases in* $[Ca^{2+}]_i$

Fifty μmol/L KA/50 μmol/L QA addition also triggered rapid increases in $[Ca^{2+}]_i$ (Fig. 9), with the elevated amplitudes being smaller than those induced by NMDA or high K^+, as revealed by the differences in the peak amplitudes of the increases in $[Ca^{2+}]_i$ shown in Figs. 3, 7, and 9. However, the QA/KA-triggered increases in $[Ca^{2+}]_i$ appeared to persist

longer at steady levels than those induced by NMDA or K^+, and did not decay following Pb^{2+} addition. These observations indicated that Pb^{2+} was not effective in blocking the QA/KA-triggered increases in $[Ca^{2+}]_i$.

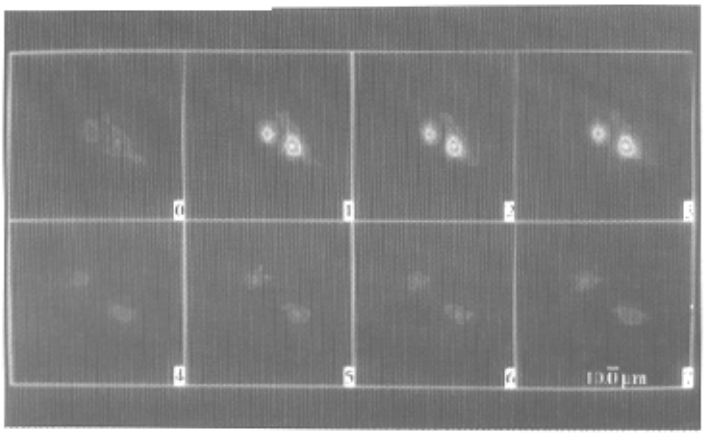

FIG. 8. Inhibitory effect of Pb^{2+} on K^+-triggered increases in $[Ca^{2+}]_i$. Two series of selected sequential images of scanned single neurons either exposed to RB (control, upper series) or 25 μmol/L Pb^{2+} containing 0.15 mol/L NaCl (lower series) about 80 s before 30 mmol/L K^+ addition were obtained. Corresponding images labelled 0 and 4, 1 and 5, 2 and 6, 3 and 7 denoted image taken just before or 20, 40, 60 s after K^+ simulation.

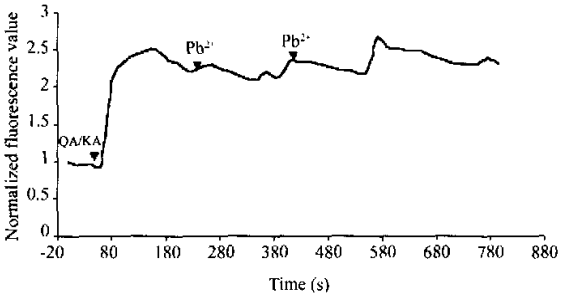

FIG. 9. Effect of Pb^{2+} on the QA/KA-activated increases in $[Ca^{2+}]_i$. $[Ca^{2+}]_i$ was scanned successively and data were expressed as normalized fluorescence values. Resting $[Ca^{2+}]_i$ was monitored for 60 s followed by 50 μmol/L QA/50 μmol/L KA stimulation (first arrow) for approximately 180 s at which time 100 μmol/L Pb^{2+} was added into the dish (second arrow). About 210 s later, 200 μmol/L Pb^{2+} was added (third arrow). Each addition of Pb^{2+} did not produce obvious influences on the QA/KA-activated increases in $[Ca^{2+}]_i$.

Effect of Pb^{2+} on Calcium Baseline

On several occasions it was noted that Pb^{2+}, in the range of 5-100 μmol/L, could slightly raise calcium levels in resting cells. This was exemplified in Fig. 10, where small

$[Ca^{2+}]_i$ increases in the resting cells were observed following the additions of 25 μmol/L Pb^{2+}. These data suggested that Pb^{2+} was able to trigger small calcium influxes into the resting neurons.

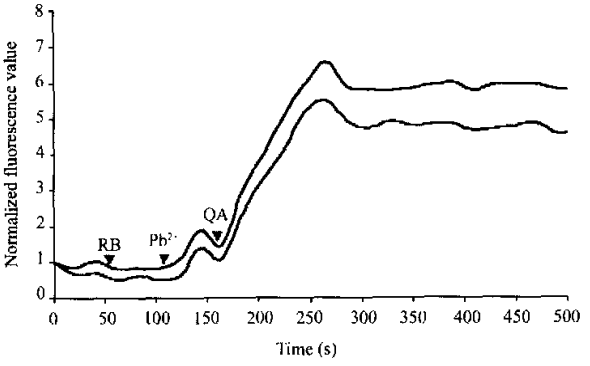

FIG. 10. Effect of Pb^{2+} on the Ca^{2+} baseline. $[Ca^{2+}]_i$ was monitored continuously and the data were expressed as normalized fluorescence values. After monitoring resting $[Ca^{2+}]_i$ levels in two cells, RB was added into the dish, having no obvious effect on the resting $[Ca^{2+}]_i$ (the first arrow). When 25 μmol/L Pb^{2+} was applied 60 s later, slight and slow increases in $[Ca^{2+}]_i$ resulted (the second arrow). The subsequent addition of 100 μmol/L QA produced a rapid increase in $[Ca^{2+}]_i$ (the third arrow).

DISCUSSION

As the neuronal functions involved in memory and learning are thought to be linked to calcium-triggered intracellular events, and hippocampus is one of the main regions underlying information storage and memory[11], $[Ca^{2+}]_i$ in cultured hippocampal neuron was measured using laser scanning confocal microscopy with the fluorescent indicator fluo-3. The results clearly demonstrated that Pb^{2+} addition either before or after NMDA/glycine stimulation inhibited the stimulated increases in $[Ca^{2+}]_i$ in the cultured neurons by interfering with the NMDA receptor channel complex. In contrast, Pb^{2+} did not significantly affect QA/KA-induced increases in $[Ca^{2+}]_i$. Thus, Pb^{2+} appeared to have a selective blocking action at the NMDA-type of glutamate receptor. In this regard, the observed action of Pb^{2+} was in agreement with previous studies[15], which reported that Pb^{2+} selectively inhibited NMDA-induced whole-cell and single-channel currents without significantly altering currents induced by either QA or KA. Since the NMDA receptor was known to play an important role in LTP which may underlie the processes for learning and memory[15, 16], this selective inhibition by Pb^{2+} may contribute to the learning disturbance. Furthermore, the above inhibitory effect of Pb^{2+} was concentration-dependent. The minimum effective concentration of Pb^{2+} observed here was 1 μmol/L which was comparable to the blood Pb^{2+} concentrations (1.5 μmol/L) of children suffering from environmental exposure to Pb^{2+}. Blood concentration of Pb^{2+} exceeding 4 μmol/L is thought to be related to acute encephalepathy[5]. Therefore, inhibition of the NMDA-receptor by Pb^{2+} may correlate with the problems caused by subclinical exposure to Pb^{2+}, as well as Pb^{2+} intoxication.

The mechanisms for Pb^{2+}-caused inhibition of NMDA receptor function remain unclear. Several previous studies suggested that Pb^{2+} might couple with a binding site on the outside

of the NMDA/glycine channel complex to modulate, but not to competitively block the channel complex[5,15]. The present study showed that raising extracellular concentrations of either NMDA from 50 μmol/L to 100 μmol/L or glycine from 10 μmol/L to 100 μmol/L after eliciting the inhibitory effect of Pb^{2+} failed to antagonize the Pb^{2+} action, suggesting that Pb^{2+} did not compete for the agonist binding sites at NMDA channels. In contrast, addition of high concentration of $[Ca^{2+}]_i$ (5 mmol/L) reversed the effect of Pb^{2+}, indicating that this effect was mediated by a competitive interaction with Ca^{2+} at the NMDA channel as in the case with AP-5. This observation was inconsistent with that of Alkondon *et al.*[15] who did not observe the same effect using the outside-out-patch configuration in cultured hippocampal neurons.

The results derived from the effects of Pb^{2+} exposure on depolarization-triggered increases in $[Ca^{2+}]_i$ in the cultured cells showed that Pb^{2+}, added before (25 μmol/L) or after (25 and 50 μmol/L) high K^+ stimulation, could prevent or reverse the triggered increases in $[Ca^{2+}]_i$, respectively. This result demonstrated that Pb^{2+} could depress voltage-activated calcium channels, adding further support to the earlier observations that Pb^{2+} acted to competitive blocking of the entry of Ca^{2+} into the terminal at neuromuscular junctions of frog and rat diaphragm via voltage-gated Ca^{2+} channels[17]. These facts will contribute to the elucidation of the mechanisms by which Pb^{2+} attenuates depolarization-evoked release of several different transmitter substances[18]. In this study we had no direct evidence bearing on how Pb^{2+} affected the voltage-gated Ca^{2+} channels.

Additionally, the current studies also indicated that Pb^{2+} could elevate the level of resting $[Ca^{2+}]_i$ in the cultured cells, an observation which could be associated with Pb^{2+} -enhanced spontaneous release of several different transmitters observed by employing various nerve tissue preparations[18].

In conclusion, Pb^{2+} modulates the actions of the NMDA receptor channel complex and voltage-activated channels causing dramatic reductions of NMDA/glycine and high K^+ responses. Our results offer a possible mechanism for an interference of Pb^{2+} with learning and cognitive development. This might explain NMDA and therefore presumably learning-related effects of prolonged exposure of the nervous system to low concentrations of Pb^{2+}.

ACKNOWLEDGEMENT

We thank Fuzhuang Wang and Aishi Ding (Academy of Military Medical Sciences) for their suggestions and technical assistance.

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(*Received September* 27, 2001 *Accepted June* 2, 2002)