Lead Blocks LTP by an Action Not at NMDA Receptors

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Exposure of children to levels of lead results in a reduction in cognitive ability and a series of behavioral deficits. We have studied the effects of PbCl₂ on long-term potentiation (LTP), the best available electrophysiological model of learning and memory, in a rat piriform cortex brain slice preparation in order to test the hypothesis that lead neurotoxicity is a result of actions on LTP. With changes in the composition of the Krebs–Ringer solution normally used in brain slices, it is possible to keep the Pb²⁺ in solution at concentrations up to 10 μM. In this concentration range, Pb²⁺ has no effect on the synaptic response elicited in piriform cortex pyramidal neurons upon stimulation of the lateral olfactory tract. We find that Pb²⁺ blocks LTP by about 75% at 5 μM and completely at 10 μM. At these concentrations, Pb²⁺ has no effect on posttetanic potentiation. Since it has been reported that Pb²⁺ blocks N-methyl-D-aspartate (NMDA) responses, and NMDA blockade is known at many sites to block LTP, we studied the effects of Pb²⁺ on NMDA responses. In the concentration range studied there was no effect of Pb²⁺ on NMDA responses. The mechanism whereby Pb²⁺ blocks LTP remains to be determined. While the concentration of Pb²⁺ found to block LTP in these studies is high relative to concentrations of Pb²⁺ in blood that are associated with causing cognitive and behavioral effects in children, the sensitivity to Pb²⁺ may be greater in young animals. © 1993 Academic Press, Inc.

INTRODUCTION

Lead neurotoxicity is a major public health problem. This is particularly so in the United States where lead-based paints were used until relatively recently, but lead is a problem throughout the world from contaminated drinking water, food stuffs, and air secondary to use of leaded gasoline, lead in water pipes and solder, and other industrial uses. Lead has no biologic function, while toxicity to the nervous system was known to the ancients (31). Recently, lead has been shown to be of particular hazard to the developing nervous system (1). Children with elevated blood lead levels, due to both pre- and postnatal exposures, show a lowered intelligence and performance on a variety of types of tasks and exhibit a shortened attention span and other behavioral abnormalities, which may be the cause of the poorer performance on IQ tests (8, 24, 25, 34). While lead has a variety of actions on biological processes, the mechanisms of lead neurotoxicity are unknown.

Long-term potentiation (LTP) is a prolonged and possibly permanent increase in synaptic excitation that follows a tetanic activation of the afferent inputs at some central nervous system sites and is one of the best available electrophysiological indicators of learning and memory (29). While the exact mechanisms of LTP are still a matter of debate, LTP at many but not all sites is blocked by substances which block N-methyl-D-aspartate (NMDA) receptors (10, 35), such as aminophosphonovaleric acid (APV) or carboxypterazine propyl-L-phosphonic acid (CPP). It has been reported that Pb²⁺ blocks NMDA responses (3) and thus it would be expected that Pb²⁺ would also block LTP via action at NMDA receptors.

We have tested this hypothesis in rat piriform cortex brain slices and find that Pb²⁺ is a potent blocker of LTP at concentrations that have no effect on responses to ionophoretically applied NMDA.

The piriform cortex brain slice preparation has considerable advantage over the more commonly studied hippocampal slice. Upon activation of the lateral olfactory tract (LOT), an APV-sensitive LTP is initiated, which remains constant after the transient posttetanic potentiation (PTP) without decrement for a period of at least 1 h (15). This preparation shows none of the short-term potentiation (14) that is characteristically seen in hippocampus and which results in a decrement of the response peak over a period of about 15 min after tetanus (16). In addition, the simple organization of the piriform cortex allows one to obtain a population response that is essentially only the population EPSP, uncontaminated by other activity (11).

METHODS

Brain slices were prepared as previously described (11). After preincubation in a standard Krebs–Ringer (composition in mM: NaCl, 126; KCl, 5; KH₂PO₄, 1.26;
MgSO₄, 1.3; CaCl₂, 2.4; NaHCO₃, 26; and glucose, 10; pH 7.3) solution, which was equilibrated with 95% O₂, 5% CO₂ for 2 h, the preparation was mounted, submerged, and perfused in the recording chamber. When lead, prepared as an aqueous solution of PbCl₂, was to be added, the preparation was perfused with a modified Krebs-Ringer solution, required in order to prevent precipitation of Pb²⁺ at physiologic pH. The modified solution had the composition (in mM): NaCl, 126; KCl, 5; MgCl₂, 1.3; CaCl₂, 2.4; glucose, 10; NaHCO₃, 6; and Tris, 3, pH 6.9. When recordings were made, this modified Krebs-Ringer (lead free) was perfused for at least 5 min prior to perfusion of the same solution containing Pb²⁺. Then after exposure to Pb²⁺, the preparation was washed again with the lead-free modified Krebs-Ringer for at least 5 min in order to assure that this manipulation had no significant effect upon the electrophysiological response. In most experiments no significant deterioration was seen if the modified Krebs-Ringer was used for less than 30 min, although more prolonged exposure often did result in reduction of response amplitudes.

The population excitatory postsynaptic potential (EPSP) response upon stimulation of the lateral olfactory tract (50 µs pulse at 5× maximal through an insulated concentric stainless-steel electrode) was recorded in the region of the distal apical dendrites, where the synaptic input terminates, with a 20-µm (1 MΩ) glass pipette filled with Krebs-Ringer (shown diagrammatically in Fig. 1, top). Because of the relative absence of interneuronal connections in this slice, the population waveform is quite smooth and is a very accurate and unobstructed reflection of the population EPSP (Fig. 1, bottom).

After stability of the response evoked by LOT stimulation at a frequency of 0.5 Hz was achieved, LTP was initiated by application of a brief (1 s at 100/s) tetanus, after which stimulation at 0.5 Hz was resumed. All recordings were digitized at 10 kHz with an IBM AT computer and data translation 2801A A-to-D converter. The initial negative peak height of the postsynaptic response was measured. In studies where lead was added, the changes in response amplitude were followed for 5 min posttetanization, but the changes in response produced by the stimulus at this frequency are known to last for at least 90 min without decrement.

The concentration of total dissolved lead in experimental Krebs-Ringer was determined by electrothermal atomisation atomic absorption spectrometry (ETA-AAS) following centrifugation to separate precipitated lead compounds. This method cannot distinguish "free" from "complexed" lead, but will definitively distinguish soluble from insoluble lead. A number of different species of lead may exist in solutions at physiologic pH. Physiologic solutions contain a number of potential ligands including the ubiquitous water molecule, hydroxide, chloride, and carbonate ions, all of which can form complexes with lead (28). It is not clear at present which or how many of these complex species have physiological activity, but it is certain that precipitated Pb²⁺ will not be active. The total dissolved lead should be viewed as that which is potentially active in a physiologic sense.

The measured lead concentrations in standard Krebs-Ringer solutions were substantially less than the nominal or total added lead. Nominal concentrations of 10 and 100 µM corresponded to measured concentrations of 2.6 ± 0.1 and 7.2 ± 1.5 µM, respectively, indicating precipitation of Pb²⁺ salts, and confirming earlier observations. Results comparing nominal and measured lead concentrations in the modified Krebs-Ringer
are presented in Fig. 2. Complete recovery of added lead was obtained in the concentration range 1.0 to 5.0 μM nominal PbCl₂. The slope of the regression was calculated to be 1.09 (95% confidence limits: 1.05–1.13), indicating a slight overestimation, which is probably due to analytical error. Above a nominal concentration of 5.0 μM, recovery was reduced, and at a nominal concentration of 20.0 μM the measured concentration was 14.45 ± 0.10 μM (72% recovery).

The modified Krebs-Ringer buffered with Tris was much more effective in preventing PbCl₂ precipitation in comparison to standard Krebs-Ringer. The reason for this is not entirely clear, but reflects increased solubility of Pb²⁺ at more acidic pH and may involve some chemical bonding between Tris and Pb²⁺.

RESULTS

Figure 3 shows a population recording of the synaptic responses from piriform cortex slices upon stimulation of the LOT and the effects of perfusion of an NMDA receptor antagonist, CPP, on the population responses and the effects of tetanic activation. The records show pen recorder records at a slow time base, such that the vertical deflections indicate the peak of the population response. This experiment was performed in normal Krebs-Ringer solution, which is being perfused at point A. At point B, CPP (10⁻⁵ M) was perfused for a period of 10 min, after which recordings were begun. It is apparent that CPP caused a slight reduction in the amplitude of the population response, indicating that a small part of the response is due to NMDA receptors. The remainder of the response is mediated through other types of excitatory amino acid receptors, as has been previously shown (7).

At point C, in the continued presence of CPP, a tetanus was applied to the LOT (100 Hz for 1 s). This stimulus caused a transient increase in the amplitude of the population response and a return to the prestimulus control value after about 100 s. This increase in response amplitude is PTP, a response seen at most synapses which is thought to result from an accumulation of calcium in the presynaptic terminal which then facilitates transmitter release (33). Point D shows the response 10 min after washing out the CPP solution with normal Krebs-Ringer, and here the response has returned to the control value. At point E a second tetanic stimulation was applied, this time in the absence of CPP. This time PTP is elicited as before, but after the decay of PTP the response remains increased relative to the pretetanus control. This increase is a reflection of LTP and is evidence that in this brain area LTP depends upon activation of NMDA receptors. The recordings at point F were obtained after 30 min of wash with normal Krebs-Ringer and show that the elevation of response does not decay over this period of time, and in fact becomes even larger. The records at point G were obtained after a second perfusion of CPP (10⁻⁵ M) for 10 min and show that the degree of reduction of the response is very similar to that observed initially at point B. The records at point H were taken 10 min after return to control Krebs-Ringer and show the maintained increase in response amplitude. LTP was consis-
about 100 s, reflecting PTP. The PTP varied in the different slices but was not significantly altered by 10 μM Pb²⁺. LTP can be seen as a maintained increase in response amplitude following the decay of PTP in the control slices, but was effectively totally blocked by Pb²⁺.

Figure 5 is a plot of the dose dependency of effects of Pb²⁺ on PTP and LTP. There was no consistent or significant effect of Pb²⁺ on PTP at any concentration, but LTP was reduced to less than one-third at 5 μM Pb²⁺ and was totally blocked at 10 μM. On the basis of previously reported results showing blockade of NMDA responses by Pb²⁺ (3), we expected to find that NMDA responses were blocked by Pb²⁺ with the same dose–response relation as is effective on LTP. However, Fig. 6 shows that Pb²⁺ (10 μM) had no effect whatsoever on ionophoretic responses to NMDA. Interestingly, the response to quisqualate (Q) was slightly increased in the presence of Pb²⁺, which is reminiscent of a potentiation of quisqualate responses previously seen with Zn²⁺ (12, 26). Ionophoretic responses to NMDA and quisqualate were studied in a total of seven neurons using Pb²⁺ concentrations between 10 and 20 μM, and in none of the neurons was there any selective blockade of NMDA responses. The potentiation of the quisqualate response was seen in several but not all cells studied.

**DISCUSSION**

Others have suggested that Pb²⁺ may block LTP in hippocampal brain slices (4) and in *in vivo* rat prepara-

![Graph showing dose dependency of blockade of LTP by PbCl₂ (n = at least 5 for each data point). LTP was totally absent at 10 and 20 μM nominal Pb²⁺ and was reduced to 30% of control in 5 μM Pb²⁺. There was no effect on PTP at any concentration.](image-url)
FIG. 6. NMDA responses in piriform cortex are not blocked by concentrations of Pb²⁺ which abolish LTP. Records are intracellular recordings from a piriform neuron upon ionophoretic application of either NMDA or quisqualate (QuiS), using methods previously described (11). There was no effect of Pb²⁺ on the NMDA response. The response to quisqualate was slightly increased in the presence of Pb²⁺.

The present studies do not allow a definitive identification of the mechanism of action of Pb²⁺ in blocking LTP. Although Pb²⁺ blocks all three types of voltage-activated Ca²⁺ channels in this concentration range in cultured dorsal root ganglion neurons (9) the present studies provide no evidence of Pb²⁺-induced reduction in PTP, known to be a result of Ca²⁺ accumulation into presynaptic terminals (27, 33). This observation implies that Pb²⁺ is not acting at these concentrations to block presynaptic calcium channels, but does not, of course, provide any information about postsynaptic calcium channels. However, there is no evidence indicating the involvement of postsynaptic channels in LTP, even though Ca²⁺ entry through NMDA-activated channels is important in at least some systems. Blockade of NMDA responses by Pb²⁺ cannot be the explanation for the effect of LTP, since responses to ionophoretic NMDA were unaffected by Pb²⁺ at concentrations that totally block LTP.

A possible mechanism of Pb²⁺ action is activation of protein kinase C (PKC). Pb²⁺ is a potent PKC activator, with a threshold concentration at 10⁻¹² M and a peak at 10⁻¹⁰ M (21). PKC has been reported [although not all authors agree (23)] to be an essential element in the induction (20) and maintenance (18) of LTP. PKC injection into hippocampal neurons elicits LTP (13). LTP induction is associated with a translocation of PKC in hippocampus (2) and translocation of PKC also occurs in classical conditioning in hippocampus (5). LTP has been reported to be no longer initiated by tetanic stimulation after PKC activation by phorbol esters (19). PKC activity in hippocampus of strains of mice that show poor spatial learning is reduced relative to strains with greater spatial learning potential (32).

Blockade of LTP by Pb²⁺ could result if PKC activity is already maximally activated by Pb²⁺ perfusion prior to the application of the tetanus. However, two observations are inconsistent with such a conclusion. The concentration range is higher than that reported to activate PKC. Furthermore, application of Pb²⁺ did not result in an increase in the amplitude of the population EPSP prior to the tetanus, as would be expected if PKC activation by Pb²⁺ were the cause of the blockade of tetanus-induced LTP.

Regardless of the mechanism by which Pb²⁺ blocks LTP there remains the important question of whether or not blockade of LTP can explain the series of symptoms of lead neurotoxicity seen in children. LTP is widely believed to be a neuronal substrate for learning and memory (29). There is also strong evidence from animal studies that the presence and magnitude of LTP are correlated with capacity for spatial learning (6).

The actual measured Pb²⁺ concentrations that we find to block LTP (5–10 µM) are much higher than blood lead concentrations associated with irreversible CNS and behavioral changes in children. However, it is difficult to compare concentrations of Pb²⁺ in perfusion media with blood lead levels, since the latter may not reflect local concentrations within the brain. There are also likely to be several different species of lead present, especially at physiological pH and concentrations of Cl⁻ (22). In addition, there is a lack of knowledge as to which species of lead are biologically active.

These experiments do not allow one to identify definitively the mechanism or site of action in the blockade of LTP by Pb²⁺, nor whether this blockade is relevant to lead neurotoxicity. It is possible that lead neurotoxicity does not depend upon LTP, or that chronic, in vivo exposure is effective in altering LTP at concentrations of Pb²⁺ lower than those effective in isolated slices. A more intriguing possibility is that lead neurotoxicity is related to blockade of LTP but that the sensitivity of LTP to Pb²⁺ is much greater within a developmental window of time. This may explain why Pb²⁺ levels that are relatively harmless in adults have such devastating effects in children. Further studies are necessary to answer these very important questions.

REFERENCES

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