FEBS Letters 377 (1995) 390–392 FEBS 16473

Pb^{2+} reduces the current from NMDA receptors expressed in *Xenopus* oocytes

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Received 10 November 1995

Abstract **We compared the effects of Pb^{2+} on four types of NMDA receptors expressed in *Xenopus* oocytes. Pb^{2+} reduced the currents evoked by glutamate and glycine. The K_i values of the receptors, $\epsilon 1/\zeta 1$, $\epsilon 2/\zeta 1$, $\epsilon 3/\zeta 1$ and $\epsilon 4/\zeta 1$, were 39, 34, 54 and 42 μM, respectively, and their Hill coefficients were 0.53, 4.6, 0.52 and 0.37, respectively. The $\epsilon 2/\zeta 1$ receptor that was inhibited in the presence of over 30 μM Pb^{2+} was not recovered to the control level after a Pb^{2+} washout for over 30 min, suggesting that $\epsilon 2/\zeta 1$ is responsible for the chronic Pb^{2+} intoxication in the nervous system.**

Key words: Glutamate receptor; *N*-Methyl-D-aspartate receptor; Pb^{2+}

1. Introduction

The NMDA subtypes of the glutamate receptor are essential for synaptic transmission and the plasticity underlying memory, learning and development of the nervous system [1–3]. The primary structures of five mouse NMDA receptor channel subunits, designated as $\varepsilon 1$, $\varepsilon 2$, $\varepsilon 3$, $\varepsilon 4$ and $\zeta 1$, have been revealed by cloning and sequencing the cDNAs [4–6]. Coexpression of each cloned $\varepsilon 1$, $\varepsilon 2$, $\varepsilon 3$ and $\varepsilon 4$ subunit together with the $\zeta 1$ subunit in *Xenopus* oocytes yields functional NMDA receptor channels. These ε subunits are distinct from each other in pharmacological properties and in situ hybridization has revealed changes in the synthesized amounts and distribution of the four ε and ζ mRNAs during the development of the mouse brain [7].

Lead is ubiquitous in the environment, where it produces not only occupational health problems in industrial workers, but may also cause chronic intoxication due to contamination in drinking water, food or air. Even a very low concentration (< 25 μg/dl, 1.2 μM) of Pb^{2+} in the blood can be the cause of an intellectual deficiency in children [8]. The critical site of its action in the brain is not obvious although a variety of actions have been demonstrated, including a blockade of the voltage-dependent calcium channels [9–11] and the NMDA receptor [12–14], as well as the inhibition of LTP [15,16]. The Pb^{2+} blockade of the NMDA receptor has been investigated in cultured fetal rat hippocampal neurons at various developmental stages and the results showed that Pb^{2+} selectively inhibits NMDA currents in young neurons [12].

Using the *Xenopus* oocyte expression sysytem, we investigated the action of Pb^{2+} on mouse NMDA receptors ($\varepsilon 1/\zeta 1$, $\varepsilon 2/\zeta 1$, $\varepsilon 3/\zeta 1$ and $\varepsilon 4/\zeta 1$) which were cloned from various development stages and various tissues in the brain.

2. Materials and methods

2.1. Plasmids

Full-length mouse NMDA receptor subunit cDNAs inserted into pBluescriptKS(+) (pBKSA$\varepsilon 1$, pBKSA$\varepsilon 2$, pBKSA$\varepsilon 3$ and pBKSA$\zeta 1$) or pSP64 (pSPGR$\varepsilon 4$) were provided by Dr. M. Mishina [4–6].

2.2. In vitro transcription

Plasmids pBKSA$\varepsilon 1$, pBKSA$\varepsilon 2$, pBKSA$\varepsilon 3$, pSPGR$\varepsilon 4$ and pBKSA$\zeta 1$ were digested with *Not*I, *Not*I, *Xba*I, *Eco*RI and *Not*I, respectively. mRNAs were synthesized in vitro from the the resulting linearized DNAs using T3 RNA polymerase for pBKSA$\varepsilon 1$, pBKSA$\varepsilon 2$, pBKSA$\varepsilon 3$ and pBKSA$\zeta 1$ and SP6 RNA polymerase for pSPGR$\varepsilon 4$ as described by Melton et al. [17,18].

2.3. Translation of mRNA in Xenopus oocytes

Stage V and VI oocytes were obtained from anesthetized *Xenopus laevis* and incubated with Ca^{2+}-free Barth medium (88 mM NaCl, 1 mM KCl, 0.33 mM $Ca(N0_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 7.7 mM Tris-HCl, pH 7.2, 18 U/ml penicillin, 18 μg/ml streptomycin) containing 2 mg/ml of collagenase at room temperature for 2 h. The follicular cell layer was then removed with forceps. The synthesized $\varepsilon 1$, $\varepsilon 2$, $\varepsilon 3$ or $\varepsilon 4$ mRNA combined with the $\zeta 1$ mRNA (the molar ratios of the $\varepsilon 1$, $\varepsilon 2$, $\varepsilon 3$ and $\varepsilon 4$ mRNAs to $\zeta 1$ mRNA were 30:1, 1:1, 1:1 and 1:1, respectively) were injected into the oocytes (5–10 ng/cell) and incubated for 2–5 days in Barth medium at 20°C before the electrophysiological study.

2.4. Electrophysiology

Currents were recorded using a conventional two-electrode voltage clamp (CA1-1a High Performance Oocyte Clamp, Dagan Corporation). The oocyte was placed in an organ bath and superfused with Mg^{2+}-free Ba^{2+} Ringer solution (115 mM NaCl, 2.5 mM KCl, 1.8 mM $BaCl_2$, 10 mM HEPES, pH 7.2) at 23–25°C. The electrodes filled with 3 M KCl had a resistance of 1–5 MΩ. Recordings were taken at a holding potential of −70 mV in Ba^{2+} Ringer solution to minimize the effect of secondarily activated Ca^{2+}-dependent Cl^- currents [19]. The currents through the NMDA receptor channels were evoked by 10 μM glutamate and 10 μM glycine in the absense or presence of Pb^{2+}. Between each series of drug additions, cells were washed with the Ringer solution for 3 min. The current signals were digitized and stored on a hard disk for later analyses. Statistics were compared by the *t* test.

The Pb^{2+} dose–response relationships for the effect on NMDA receptors were determined by fitting the peak values of currents to the Langmuir equation:

$$I[Pb^{2+}] = I(\text{control})(1/(1 + [Pb^{2+}]^n/K_i),$$

where $I[Pb^{2+}]$ is the current measured in the presence of a given concentration of Pb^{2+}, I(control) is the current before the addition of Pb^{2+}, K_i is the apparent dissociation constant and n is the Hill coefficient.

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Abbreviations: NMDA, *N*-methyl-D-aspartate: LTP, long-term potentiation; APV, DL-2-amino-5-phosphonovalerate.

SSDI 0014-5793(95)01402-0

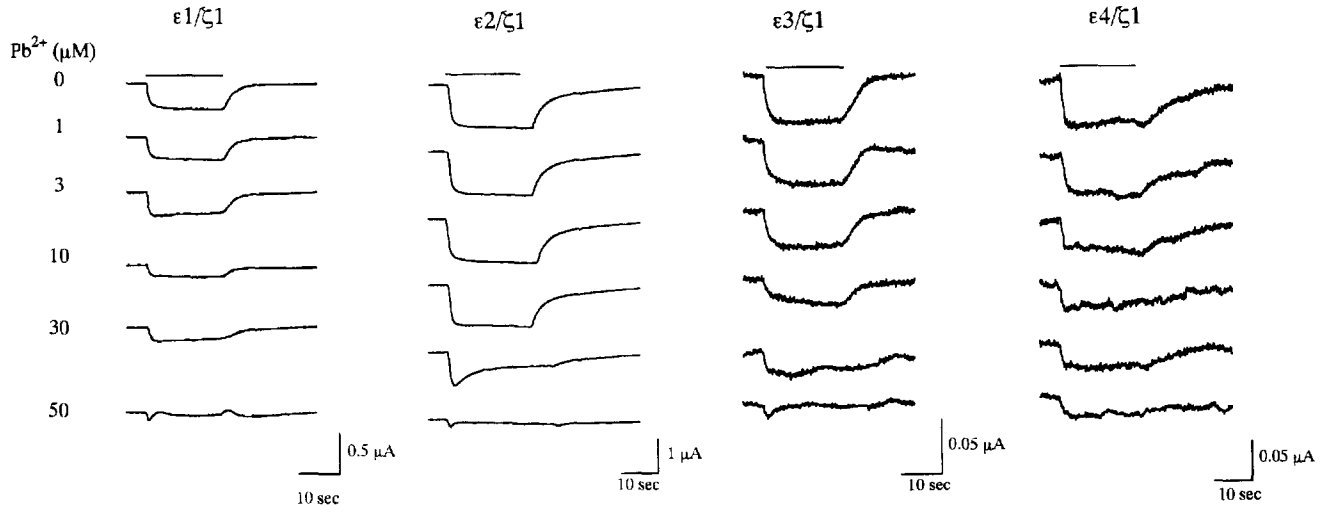

Fig. 1. Effects of Pb^{+2} on four types (ε1/ζ1, ε2/ζ1, ε3/ζ1 and ε4/ζ1) of NMDA receptors expressed in *Xenopus* oocytes. Bars above traces indicate the length of the glutamate (10 μM) and glycine (10 μM) pulses.

3. Results and discussion

3.1. Characterization of NMDA receptors

Receptors of all the four types expressed in oocytes evoked currents upon stimulation by 100 μM NMDA and 10 μM glycine. The effects of APV (100 μM) and Zn^{2+} (3 μM) on the currents were also examined to identify the NMDA receptor subtypes expressed. The sensitivities to APV and Zn^{2+} were in the order ε1/ζ1 > ε2/ζ1 > ε3/ζ1 > ε4/ζ1 and ε1/ζ1 < ε2/ζ1 < ε3/ ζ1 < ε4/ζ1, respectively, consistent with earlier reports [20,21] (data not shown). Among the receptors, ε2/ζ1 was most sensitive to Zn^{2+} and the ε4/ζ1 receptor was most sensitive to Pb^{2+} in this present study. Ujihara et al. reported that the mechanisms of the inhibition by Zn^{2+} and Pb^{2+} were different [12]. These results indicated that Pb^{2+} and Zn^{2+} interact with different binding sites.

3.2. Effects of Pb^{2+}

The sensitivity to Pb^{2+} of the ε/ζ heteromeric channels expressed in oocytes was examined by measuring the peak currents evoked by L-glutamate and glycine (Fig. 1). ε1/ζ1, ε2/ζ1 and ε3/ζ1 receptor channels were densensitized at Pb^{2+} concentrations exceeding 30 μM. The effect of Pb^{2+}on the NMDA receptors is summarized in Fig. 2. At 1 μM Pb^{2+}, the ε1/ζ1 and ε4/ζ1 receptors were inhibited but ε2/ζ1 was not. At 100 μM, all four receptors completely lost the channel activities (data not shown). The K_i values for the blocking action of Pb^{2+} of ε1/ζ1, ε2/ζ1, ε3/ζ1 and ε4/ζ1 receptors were 39, 34, 54 and 42 μM, respectively, and their Hill coefficients were 0.53, 4.6, 0.52 and 0.37, respectively. The high Hill coefficient of the ε2/ζ1 receptor suggested that the receptor has multiple Pb^{2+}-binding sites. After exposure to excess Pb^{2+} (30 μM), the current of the ε2/ζ1 receptor did not return to the control level even after a washout for over 30 min (Fig. 3). Busselberg et al. have also reported the similar sensitivity of Pb^{2+} to NMDA receptor in neurons acutely dissociated from the hippocampus in which the K_i and the Hill coefficient were 45 μM and 1.1, respectively [14]. The ε1, ε2 and ζ1 subunits are present in hippocampal neurons [7]. The results may reflect mixed currents from the ε1/ζ1 and ε2/ζ1 receptors. The effect of Pb^{2+} on the receptors was not voltage-dependent (data not shown), as found in neurons acutely dissociated from the hippocampus [14]. The irreversib-

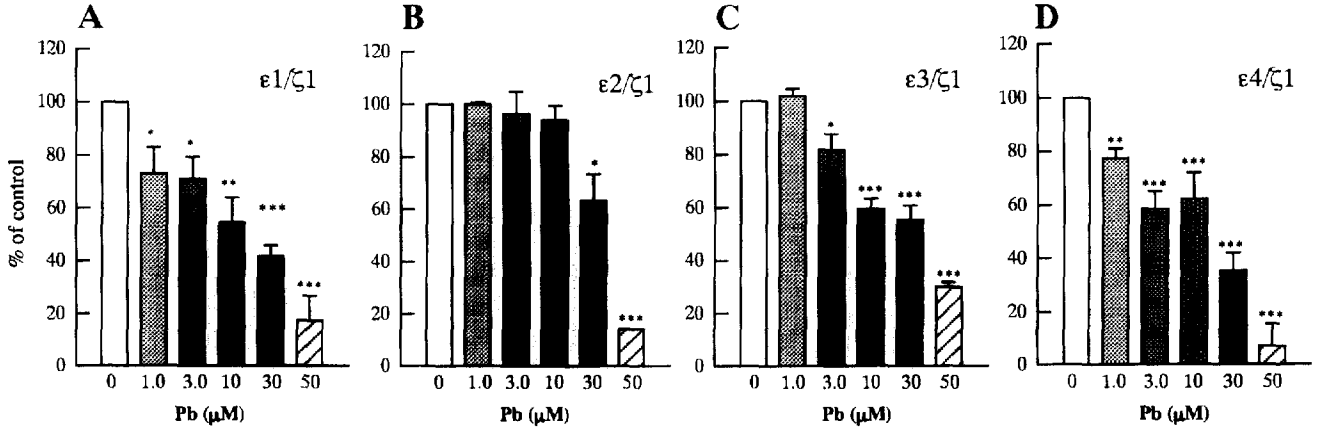

Fig. 2. Summary of the effects of Pb^{2+} on the NMDA receptors. (A)ε1/ζ1 receptor (B) ε2/ζ1 receptor, (C) ε3/ζ1 receptor and (D) ε4/ζ1 receptor. Results are presented as mean ± S.E.M. (n = 3–7). (*P < 0.05, **P < 0.01, ***P < 0.001, t test compared with controls untreated by Pb^{2+}.)

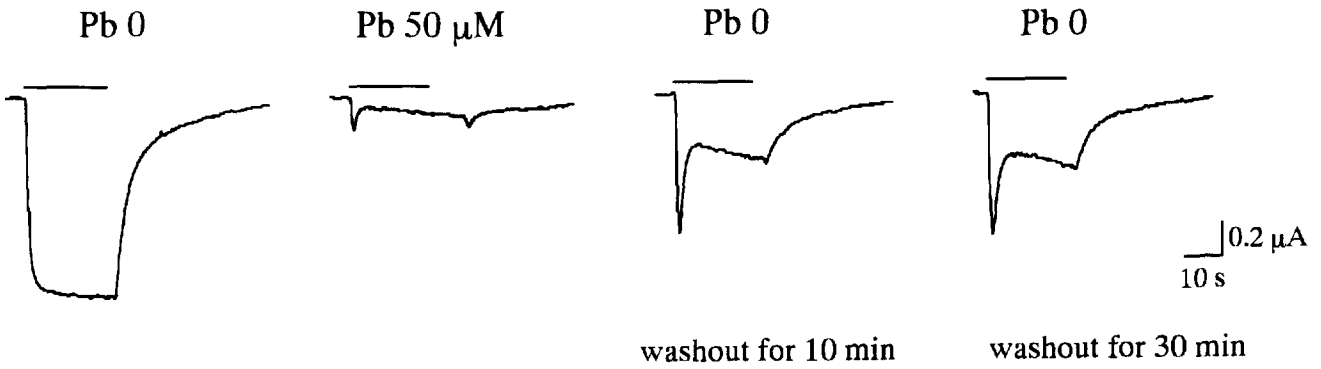

Fig. 3. Irreversible effect of Pb^{2+} on $\varepsilon 2/\zeta 1$-elicited currents. Bars above traces indicate the length of glutamate (10 μM) and glycine (10 μM) pulses. The application of 50 μM Pb^{2+} reduced the currents immidiately and they did not recover to the control level even after a washout for 30 min.

lity of the Pb^{2+} effect on the $\varepsilon 2/\zeta 1$ receptor would explain the chronic Pb^{2+} intoxication in the nervous system. Mice lacking the $\varepsilon 1$ subunit have reduced hippocampal LTP and spatial learning [22]. The $\varepsilon 1/\zeta 1$ receptor is postnatally expressed and widely distributed in the brain and $\varepsilon 4/\zeta 1$ receptors are present in the diencephalon and brainstem from the embryonic, through the neonatal stage [7]. Both the receptors were inhibited by Pb^{2+} at the low concentration shown in the present experiment. Therefore, the high sensitivity of $\varepsilon 1/\zeta 1$ and $\varepsilon 4/\zeta 1$ receptors to Pb^{2+} may be responsible for the learning difficulties seen in children who are exposed to low levels of Pb^{2+}.

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