Zn\(^{2+}\) Blocks the NMDA- and Ca\(^{2+}\)-Triggered Postexposure Current \(I_{\text{pe}}\) in Hippocampal Pyramidal Cells

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INTRODUCTION

N-methyl-D-aspartate (NMDA) exposure can lead to the activation of a cation current called the postexposure current \((I_{\text{pe}})\) in hippocampal neurons (Chen et al. 1997). The conductance underlying \(I_{\text{pe}}\) shows a high Ca\(^{2+}\) permeability. \(I_{\text{pe}}\) is triggered by the increase in intracellular Ca\(^{2+}\) concentration caused by Ca\(^{2+}\) influx through the NMDA receptor channel. Once triggered, \(I_{\text{pe}}\) continues to increase in amplitude (in the absence of NMDA) until death of the neuron occurs. Procedures that prevent the induction of \(I_{\text{pe}}\) or suppress it after induction also reduce neuronal death (Chen et al. 1997). This paper identifies Zn\(^{2+}\) as an effective blocker of \(I_{\text{pe}}\). The availability of a blocker should facilitate further studies into the intracellular activation pathway of \(I_{\text{pe}}\) and the role of \(I_{\text{pe}}\) in NMDA toxicity.

METHODS

Acutely isolated hippocampal CA1 neurons from adult guinea pigs were prepared according to the Kay and Wong (1986) method with several modifications to increase the harvest of healthy neurons and preserve NMDA responses (see Chen et al. 1997). Healthy neurons were selected by choosing those that were uniformly bright under phase contrast microscopy. These neurons have a normal (around \(-60 \text{ mV}\)) and stable resting potential within the first hour of recording, have an ability to fire action potentials, and show reversible receptor-channel modulation by second messenger systems (Chen and Wong 1995a,b; Chen et al. 1990).

Whole cell voltage-clamp was performed following the procedure described by Hamill et al. (1981) with the use of a List EPC-7 patch-clamp amplifier and pClamp software (Axon Instruments). Access resistances were \(-10 \Omega\). Good recordings were ensured by discarding cells that had seal resistances <20 G\(\Omega\), which took more than four gentle sucks to break the membrane, or which did not maintain a stable input resistance during the first 5 min of recording before NMDA exposure. The holding potential was \(-50\) or \(-55 \text{ mV}\).

Cells were in a 1-ml bath that was perfused continuously with extracellular control solution at a rate of \(1-2 \text{ ml per min}\). Extracellular control solution contained (in mM) 140 NaCl, 2 KCl, 2 CsCl, 10 HEPES, pH adjusted to 7.2 with methanesulfonic acid (M). Prolonged Zn\(^{2+}\) block (Fig. A) shows that the membrane current returned to baseline after NMDA exposure and cell death. \(I_{\text{pe}}\) was blocked during short applications of Zn\(^{2+}\) (25 s, 500 \(\mu\text{M}\)) but otherwise continued to grow in amplitude once it was triggered. The conductance associated with \(I_{\text{pe}}\) was greatly reduced during the Zn\(^{2+}\) block (Fig. B). A shorter (2 min) application of NMDA could also activate \(I_{\text{pe}}\), but there was a delay after the end of the NMDA application before \(I_{\text{pe}}\) was evident (Fig. B). The \(I_{\text{pe}}\) triggered by these shorter applications of NMDA was also blocked during short applications of Zn\(^{2+}\) (Fig. B).

All twelve cells tested with a 10 min NMDA exposure developed \(I_{\text{pe}}\). In all twelve cases, the \(I_{\text{pe}}\) became evident during the NMDA exposure. Eight of 10 cells tested with a 2-min NMDA exposure developed \(I_{\text{pe}}\). In these eight cells, the membrane current returned to baseline after NMDA exposure and \(I_{\text{pe}}\) became evident after a \(7 \pm 3 \text{ min}\) (mean ±

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Zn\textsuperscript{2+} BLOCKS THE POSTEXPOSURE CURRENT \(I_{pe}\)

FIG. 1. Postexposure current (\(I_{pe}\)) activated by NMDA exposure or intracellular calcium perfusion is blocked by brief pulses of Zn\textsuperscript{2+}. A: \(I_{pe}\) triggered by a 10 min N-methyl-D-aspartate (NMDA) exposure was blocked during brief (25 s) pulses of Zn\textsuperscript{2+} (500 \(\mu\)M). Zn\textsuperscript{2+} application had no effect on holding current before NMDA exposure. B: Zn\textsuperscript{2+} pulses. Voltage steps (-10 mV, 25 s) demonstrate increase in membrane conductance associated with \(I_{pe}\). Zn\textsuperscript{2+} was again blocked during brief (20 s) pulses of Zn\textsuperscript{2+} (500 \(\mu\)M). C: \(I_{pe}\) triggered by intracellular perfusion of high calcium solution was blocked during brief (20 s) pulses of Zn\textsuperscript{2+} (500 \(\mu\)M). Holding potential was -50 mV.

The proportion of cells developing \(I_{pe}\) further fell to 4 of 12 cells and 1 of 10 cells exposed to 40 s and 10 s of NMDA, respectively. In the four cells that developed \(I_{pe}\) after a 40 s NMDA exposure, \(I_{pe}\) became evident after a 10 ± 4 min delay. In the one cell that developed \(I_{pe}\) after a 10 s exposure, \(I_{pe}\) became evident after a 12-min delay. In all cases in which \(I_{pe}\) developed, it was blocked during short pulses of Zn\textsuperscript{2+} (20–25 s, 100–500 \(\mu\)M).

In the earlier study (Chen et al. 1997) we showed that a cation current with the properties of \(I_{pe}\) could also be triggered by intracellular perfusion of Ca\textsuperscript{2+}. Figure 1C shows that the current triggered by intracellular perfusion of high calcium solution was also blocked during short applications of Zn\textsuperscript{2+} (500 \(\mu\)M, \(n = 8\)).

Additional experiments were done to test the ability of various divalent cations to block \(I_{pe}\) (Fig. 2A). Cd\textsuperscript{2+} (1 mM) and Zn\textsuperscript{2+} (100 \(\mu\)M) reduced \(I_{pe}\) by 90 ± 6% (mean ± SD, \(n = 25\)) and 88 ± 5% (\(n = 21\)), respectively. Co\textsuperscript{2+} (1 mM) and Mn\textsuperscript{2+} (1 mM) reduced \(I_{pe}\) by 17 ± 4% (\(n = 7\)) and 5 ± 3% (\(n = 4\)), respectively. Mg\textsuperscript{2+} (1 mM, \(n = 8\)) and Ba\textsuperscript{2+} (1 mM, \(n = 6\)) had no effect. Figure 2C illustrates the dose-response curve for Zn\textsuperscript{2+}. Some suppression of \(I_{pe}\) was apparent at 5 \(\mu\)M and the effect saturated at 500 \(\mu\)M. Between these two concentrations the effect of Zn\textsuperscript{2+} showed a steep concentration dependence. The IC\textsubscript{50} of Zn\textsuperscript{2+} (the concentration of Zn\textsuperscript{2+} at which 50% of maximal block was achieved, see Fig. 2) was 64 \(\mu\)M. Cd\textsuperscript{2+} had an IC\textsubscript{50} of ~350 \(\mu\)M.

Because \(I_{pe}\) is triggered by Ca\textsuperscript{2+} and partially carried by Ca\textsuperscript{2+} (Chen et al. 1997), we suspected that \(I_{pe}\)-mediated Ca\textsuperscript{2+} influx might be responsible for the continuous growth of \(I_{pe}\). Figure 3B illustrates a prolonged block of \(I_{pe}\) with 100 \(\mu\)M Zn\textsuperscript{2+}. Brief pulses of Zn\textsuperscript{2+}-free solution were applied once every min to test the development of \(I_{pe}\) during the prolonged Zn\textsuperscript{2+} block. If the hypothesis were incorrect and cation influx through \(I_{pe}\) were not required for the further growth of \(I_{pe}\), one would expect the Zn\textsuperscript{2+}-free pulses to reveal a continuous growth in the underlying \(I_{pe}\) similar to that seen in the control (Fig. 3A). Instead, in support of the hypothesis, the \(I_{pe}\), which was continuously growing before...
Zn$^{2+}$ block, was maintained at nearly a constant amplitude during the 10-min Zn$^{2+}$ block. The experiment was performed 10 times with either 100 μM Zn$^{2+}$ or 1 mM Cd$^{2+}$. Six of 10 cells showed a ≈5% change in the amplitude of the underlying $I_{\text{pc}}$ during the prolonged block and 4 of 10 showed a 75% or greater reduction in the rate of increase of $I_{\text{pc}}$ as compared with the rate at which it was increasing before the Zn$^{2+}$ (or Cd$^{2+}$) block. When the Zn$^{2+}$ (or Cd$^{2+}$) was removed, the growth in $I_{\text{pc}}$ resumed.

**Discussion**

Ca$^{2+}$ and Na$^+$ influx associated with $I_{\text{pc}}$ may be the cause of NMDA-triggered neuronal toxicity in acutely isolated hippocampal neurons (Chen et al. 1997). In fact, in our studies the development of $I_{\text{pc}}$ is diagnostic for whether or not a cell will die because of NMDA exposure: those cells that develop $I_{\text{pc}}$ (and are allowed to express $I_{\text{pc}}$) die in <1 h; those that do not develop $I_{\text{pc}}$ die at the same rate as cells that were not exposed to NMDA (2–4 h).

This paper establishes Zn$^{2+}$ as an effective blocker of $I_{\text{pc}}$. The saturation of the Zn$^{2+}$ effect at a submillimolar concentration supports the hypothesis that Zn$^{2+}$ is binding to a specific receptor site. Having a blocker of $I_{\text{pc}}$ available allowed us to confirm and extend findings made in the previous study. Our earlier results showed that extracellular Ca$^{2+}$ is required for the continuous growth of $I_{\text{pc}}$ but not for the maintenance of a steady $I_{\text{pc}}$ (Chen et al. 1997). These earlier results combined with the finding that $I_{\text{pc}}$ remained at a steady level during prolonged Zn$^{2+}$ block indicate that once triggered, the maintenance of a steady $I_{\text{pc}}$ is not dependent on $I_{\text{pc}}$-mediated Ca$^{2+}$ influx but that the continuous growth in $I_{\text{pc}}$ is dependent on $I_{\text{pc}}$-mediated Ca$^{2+}$ influx.

Zn$^{2+}$ is present in many axon terminals and can be released during neuronal activity (Assaf and Chung 1984; Howell et al. 1984). During intense neuronal activity, which may be expected to be associated with a large level of glutamate release, the Zn$^{2+}$ concentration in the extracellular space of rat hippocampus was estimated to peak at 300 μM (Assaf and Chung 1984), which would be a sufficient concentration to suppress $I_{\text{pc}}$. Earlier studies have shown that extracellular Zn$^{2+}$ can attenuate NMDA toxicity by blocking the NMDA receptor channel (Peters et al. 1987) but, on the other hand, can kill cells at higher concentrations (Choi et al. 1988). $I_{\text{pc}}$ block is another avenue by which low concentrations of extracellular Zn$^{2+}$ might help protect neurons from cell death.

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