INHIBITION OF [3H]GLUTAMATE RELEASE BY Zn\(^{2+}\) IN RAT HIPPOCAMPAL SLICES

Tetsuroh KIHARA,* Akemichi BABA, Yutaka KOYAMA, ** Takafumi ISHIHARA * and Heitaroh IWATA **

Laboratory of Molecular pharmacology, Suntory Institute for Biomedical Research*, 1-1-1 Shimamoto-cho, Mishima-gun, Osaka 618, Japan and Department of Pharmacology, Faculty of Pharmaceutical Sciences, Osaka University**, 1-6 Yamada-oka, Suita, Osaka 565, Japan

Zn\(^{2+}\) at the concentration of 10 \(\mu\)M inhibited the depolarization-induced [3H]glutamate release from the preloaded rat hippocampal slices both in the presence and absence of Ca\(^{2+}\) without affecting [3H]GABA and [3H]ACH release. Of divalent cations tested, Zn\(^{2+}\) and Fe\(^{3+}\) had an inhibitory effect on the release of glutamate.

KEYWORDS—glutamate release; rat hippocampal slice; Zn\(^{2+}\)

INTRODUCTION

Zinc (Zn\(^{2+}\)), an essential trace element, is highly concentrated in the giant boutons of hippocampal mossy fibers. It has been shown that the uptake and release of Zn\(^{2+}\) in hippocampal slices are regulated by the neuronal activity of mossy fibers. Exogenously applied Zn\(^{2+}\) inhibits mossy fiber synaptic transmission and has an antagonistic effect on N-methyl-D-aspartic acid (NMDA) receptors, a subclass of excitatory amino acid receptors. Thus, Zn\(^{2+}\) seems to be involved in mossy fiber synaptic transmission. Although the neurotransmitter of the mossy fiber pathway is not yet defined, most studies suggest that an excitatory amino acid, for example glutamate, is a candidate for the mossy fiber. Based on these findings, it might be expected that Zn\(^{2+}\) and presumably glutamate are released from mossy fiber terminals by depolarization. It is not known how Zn\(^{2+}\) affects the uptake and release of glutamate in nerve endings. In the study to clarify the functional relevances of the excitatory amino acid neurons with Zn\(^{2+}\) in hippocampus, we found that dithizone, a Zn\(^{2+}\) chelator, preferentially depleted glutamate in the hippocampus. In addition, we have recently found that glutamate

*** To whom correspondence should be addressed.
increased the dissociation of Zn$^{2+}$ from its soluble proteins of the hippocampus.\textsuperscript{10} To elucidate functional roles of Zn$^{2+}$ in neurons with glutamate, the present study shows that Zn$^{2+}$ selectively inhibited the release of [$^3$H]glutamate from the preloaded hippocampus.

MATERIALS AND METHODS

Release experiments by the superfusion method were performed as described previously\textsuperscript{11} with a slight modification. Male Sprague-Dawley rats weighing 120-200 g were used throughout. Hippocampal slices (0.25 mm thick) were preincubated in Krebs-Ringer buffer (KR), which was continuously bubbled with 95% O$_2$-5% CO$_2$ at 37 °C for 20 min (about 80 mg slices/20 ml KR). KR contained in mM: NaCl, 138; KCl, 5.6; CaCl$_2$, 1.0; NaH$_2$PO$_4$, 1.0; NaHCO$_3$, 11; D-Glucose, 10; HEPES, 20 (pH 7.4). When Zn$^{2+}$ was added to the medium, NaHCO$_3$ was eliminated from KR to avoid the formation of ZnCO$_3$. The slices were preloaded with either [$^3$H]glutamate (6 μCi/2 ml) or [$^3$H]γ-aminobutyric acid (GABA) (4 μCi/2 ml) for 10 min. For [$^3$H]acetylcholine (ACh) release, slices were preloaded with [$^3$H]choline (4 μCi/2 ml) for 15 min. The preloaded slices were washed and transferred to the superfusion chambers. Slices were superfused at the high rate (2 ml/min) to minimize reuptake of released [$^3$H]glutamate.\textsuperscript{11} Aminoxyacetic acid (0.1 mM) and eserine (0.2 mM) were added to the KR for the release of [$^3$H]GABA and [$^3$H]ACh release, respectively. Each superfusate was collected to the test tubes with a 2 min interval and its radioactivity was determined. Release was expressed as the percentage of the total radioactivity taken up by the tissues. The evoked release by high K$^+$ or veratrine was carried out by exchanging the normal KR to the medium containing 40 mM KCl or 7 μg/ml veratrine. Na$^+$ -dependent and Cl$^-$ -dependent [$^3$H]glutamate uptakes by synaptic membrane vesicles were performed as described elsewhere.\textsuperscript{11,12}

Statistical changes were analyzed by Student's $t$ test. L-[$^3$H]glutamic acid (39 Ci/mmol) and [$^3$H]GABA (78 Ci/mmol) were purchased from the Radiochemical Centre, Amersham, Bucks, U.K.. [$^3$H]Choline (80 Ci/mmol) was obtained from New England Nuclear, Boston, MA, USA.

All other reagents were the highest analytical grade available.

RESULTS AND DISCUSSION

Effect of Zn$^{2+}$ on high K$^+$ -evoked release of [$^3$H]glutamate, [$^3$H]GABA and [$^3$H]ACh from the preloaded hippocampal slices were investigated. As shown in
Inhibition of Glutamate Release by Zn^{2+}

Fig. 1A, Zn^{2+} at the concentrations of 10 and 100 μM inhibited high K\(^{+}\) - induced [\(^{3}\)H]glutamate release by 46.9% and 48.3%, respectively. However, 100 μM Zn\(^{2+}\) did not affect the release of [\(^{3}\)H]GABA or [\(^{3}\)H]ACH at the same concentration (Fig. 1B). Zn\(^{2+}\) also showed the substantial inhibition of [\(^{3}\)H]glutamate at the concentration of 100 μM without affecting the release of [\(^{3}\)H]GABA and [\(^{3}\)H]ACH. In these experiment, Zn\(^{2+}\) did not affect the spontaneous release of [\(^{3}\)H]glutamate, [\(^{3}\)H]GABA and [\(^{3}\)H]ACH (data not shown). In the present incubation condition, 72.1% of the released radioactivity in the medium, and 57.4% of the radioactivity uptaken by the tissues have been demonstrated to be that of [\(^{3}\)H]glutamate by using high voltage paper electrophoresis. In the present condition, high K\(^{+}\) - evoked [\(^{3}\)H]glutamate release under Ca\(^{2+}\) -free KR was 61.7% of that under normal KR. Zn\(^{2+}\) at the concentration of 10 μM inhibited high K\(^{+}\) -induced [\(^{3}\)H]glutamate release in Ca\(^{2+}\) -free KR by 72.2% (Fig. 1C).

![Graph A](image1)

![Graph B](image2)

![Graph C](image3)

Fig. 1. Effect of Zn\(^{2+}\) on Depolarization - Induced Release of [\(^{3}\)H]Glutamate, [\(^{3}\)H]GABA and[\(^{3}\)H]ACH from Preloaded Rat Hippocampal slices

A. High K\(^{+}\) (40 mM KCl)-induced release. * P < 0.05, ** P < 0.01 compared with Control (white column). B. Veratrine (7 μ g/ml)-induced release. *** P<0.01 compared with Control (white column). C. High K\(^{+}\) -induced [\(^{3}\)H]glutamate release in Ca\(^{2+}\) -free medium. Values are mean ± S.E. of 4 to 7 separate experiments.
Effect of some divalent cations in high K⁺-evoked release of [³¹]H-glutamate from the preloaded hippocampal slices was investigated (Table I). The inhibition of glutamate release by Zn²⁺ was relatively specific, since other divalent cations except for Zn²⁺ and Fe²⁺ had no significant effect. Although a high amount of Fe²⁺ has been found in the central nervous system,¹⁴,¹⁵ it is uncertain whether Fe²⁺ has specified neuronal significance. At present, it is difficult to assess the physiological significance of the Fe²⁺-induced inhibition of glutamate release. It may be that Fe²⁺ attenuates the release of glutamate in the same manner as Zn²⁺ does.

In our separate experiment, we have found that high K⁺-evoked uptake of ⁴²Ca²⁺ to crude synaptosomal fraction of hippocampus was not influenced by 10 μM Zn²⁺ (data not shown). Moreover, Co²⁺ and Ni²⁺, which have a Ca²⁺ channel antagonist-like action in rat brain synaptosomes,¹⁶ did not inhibit the release of [³¹]H-glutamate in the present study (Table I). Actually, the inhibition of [³¹]H-glutamate release by Zn²⁺ was Ca²⁺-independent, and so Zn²⁺ is not likely to interact with the process of excitatory-secretion coupling in glutamate neurons. Ca²⁺-independent release of glutamate and GABA on synaptosomal depolarization is due to the thermodynamic reversal of the plasma membrane Na⁺-cotransport system.¹⁷ To examine this possibility, we studied the effect of Zn²⁺ on Na⁺-dependent and Cl⁻-dependent glutamate transports in synaptic membrane vesicles. Results showed that no significant effect of Zn²⁺ was observed on both transports at the concentration range of 1 to 100 μM (data not shown). Since Zn²⁺ had no effect on Na⁺-dependent glutamate transport, the inhibition of [³¹]H-glutamate by Zn²⁺ is not related to apparent uptake of glutamate in the perfusion condition. From all of the evidence, it is not likely that Zn²⁺ inhibits the release of glutamate by interacting with Na⁺-cotransport system. Although the inhibitory mechanism of Zn²⁺ on [³¹]H-glutamate release is yet unknown, the lack of the effect of Zn²⁺ on [³¹]H-GABA release may indicate a specificity of the action on glutamate neurons. The concentration of Zn²⁺ (10 μM) is not high compared to that in the synaptic cleft after depolarization.¹⁸ Zn²⁺, which localizes in hippocampal mossy fiber terminals, is released by excitation¹⁹ and thus Zn²⁺ has a modulatory effect on the transmission of glutamate neurons in hippocampus.
Table I. Effect of Divalent Cations on High K⁺-Induced [³H]Glutamate Release from the Preloaded Rat Hippocampal Slices

<table>
<thead>
<tr>
<th>Divalent cations (100 μM)</th>
<th>High K⁺-induced [³H]glutamate release (% Release)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.53 ± 0.46</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>2.71 ± 0.34 **</td>
</tr>
<tr>
<td>Co²⁺</td>
<td>4.18 ± 0.81</td>
</tr>
<tr>
<td>Fe²⁺</td>
<td>3.38 ± 0.29 *</td>
</tr>
<tr>
<td>Mn²⁺</td>
<td>3.53 ± 0.84</td>
</tr>
<tr>
<td>Ni²⁺</td>
<td>6.11 ± 1.09</td>
</tr>
</tbody>
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Values are means ± S.E.M. of 4 experiments.  * P<0.05,  ** P<0.01 compared with Control.

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