Comparison of the L-Glutamate Level in Mouse Hippocampal Slices under Tetraethylammonium Chloride Stimulation as Measured with a Glass Capillary Sensor and a Patch Sensor

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The concentration level of L-glutamate released from the region CA1 of mouse hippocampal slices under tetraethylammonium chloride (TEA) stimulation was measured by two independent methods, i.e., a glass capillary-based enzyme sensor and a patch sensor, and compared with each other for different slice preparations. In a submerged slice preparation, the sensors were positioned in bath solutions several tens μm above CA1, respectively. The sensors exhibited almost the same level of extra-slice L-glutamate concentration. When a capillary sensor was implanted in region CA1 at a depth of approximately 10 μm, the TEA-induced L-glutamate release pattern was very similar to those observed with the capillary sensor in a bath use. The concentration level of intra-slice (extracellular) L-glutamate was found to be in the range from 6 to 10 μM, which was significantly larger than that of the extra-slice one. These results demonstrate that L-glutamate released from each neuronal region inevitably diffuses out of the slices, and the extra-slice L-glutamate level reflects the extracellular one.

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Introduction

L-Glutamate is a major amino acid neurotransmitter in the central neuronal system of mammalian brain, and plays a vital role in brain development, synaptic plasticity, neurotoxicity, and neuropathological disorders.1-6 The basal and enhanced levels of extracellular L-glutamate play a key role in neuronal functions, because its level will determine whether L-glutamate has actions or negligible actions on most glutamate receptors.2 Despite technical limitations, progress has been made in sensing L-glutamate in vivo and in vitro with microsensors,5,9 such as microdialysis electrodes,10-12 glass capillary-based enzyme sensors,13-15 polymer-coated enzyme sensors16-18 and patch sensors based on natural sensing probes,19-21 which possess the spatial and temporal resolution necessary for discriminating the regional distribution of L-glutamate.

From a methodological point of view, there are two technical ways to monitor L-glutamate release in acute brain slices.9 One approach uses a microsensor implanted into the vicinity of a target neuronal region to monitor extracellular glutamate. In the other approach, a microsensor is dipped into a bath solution at a given distance above the target neuronal region of a submerged slice, and monitors L-glutamate that diffuses out of the slice. The in vitro “extra-slice” measurements with microsensors in the bath use revealed that the concentration level of L-glutamate in acute brain slices released by chemical stimulation are markedly dependent on the neuronal regions and the types of stimulation.13,14 On the other hand, in vitro and in vivo “intra-slice” measurements allowed real-time monitoring of extracellular L-glutamates in acute brain slices15,16 and in brains,17-21 respectively. However, the reported basal and enhanced level of extracellular L-glutamate varied over a wide range,9 seemingly because the methods for calibrating the sensor response are not yet established.

In the present work, considering the importance of knowing the regional distribution of L-glutamate in acute hippocampal slices, we monitored L-glutamate release in region CA1 with two independent sensors, i.e. a glass capillary-based enzyme sensor13,14 and a patch sensor.29 The hippocampal slices of submerged and interface preparations were stimulated by tetraethylammonium chloride (TEA) as a stimulant. The extra-slice concentration level of L-glutamate obtained with a capillary sensor was compared with that obtained with a patch sensor in order to demonstrate the accuracy of each method. Furthermore, the extra-slice L-glutamate level was compared with that obtained with an implanted capillary sensor to understand whether the concentration level of L-glutamate that diffused out of acute slices into a bath solution reflected the extracellular one.

Experimental

Reagents

L-Glutamate oxidase (GluOx) from streptomyces sp. X-119-6 (6.3 U/mg powder) was obtained from Yasama Shouyu (Chosi, Japan). Ascorbate oxidase (from curcurbita sp. 210 U mg powder), L-glutamic acid and 10% (v/v) glutaraldehyde solution were obtained from Wako Pure Chemicals Co. (Osaka, Japan). Bovine serum albumin (BSA), horseradish peroxidase (HRP) and TEA were purchased from Sigma Chemical Co. (St Louis, MO). Piperazine-1,4-bis(2-ethanesulfonic acid) (HEPES) was obtained from Dojindo Laboratories (Kumamoto, Japan). An

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osmium poly(vinylpyridine) redox polymer mediator solution containing horseradish peroxidase (Os-gel-HRP) was obtained from Bioanalytical Systems Inc. (NAS, West Lafayette, IN). All other chemicals used were all of analytical reagent grade. Milli-Q water (Millipore reagent system, Benford, MA) was used throughout the experiments.

An artificial cerebrospinal fluid (ACSF) contained 0.12 M NaCl, 3.0 mM KCl, 2.0 mM CaCl₂, 2.0 mM MgSO₄, 23 mM NaHCO₃, 1.2 mM NaH₂PO₄ and 11 mM d-glucose, saturated with a 95% O₂/5% CO₂ gas mixture. A Ca²⁺ and Mg²⁺-free ACSF was prepared and used as an inner solution of a glass capillary electrode. A TEA solution contained 95 mM NaCl, 5.0 mM KCl, 5.0 mM CaCl₂, 0.10 mM MgCl₂, 24 mM NaHCO₃, 1.25 mM KH₂PO₄, 10 mM d-glucose and 25 mM TEA, saturated with a 95% O₂/5% CO₂ mixture. An L-glutamate solution was prepared by dissolving L-glutamic acid in an ACSF.

Slice preparation
Adult male ddY mice (7-week-old) were decapitated under ether anesthesia. Coronal slices (thickness 250 or 300 μm) were cut using a Dosaka DTK-1000 microslicer (Kyoto, Japan). The slices were incubated for 30 min in an ACSF at 30 - 32°C and held at room temperature until use. For interface slice preparation, hippocampus was taken out from brain slices and transferred on a lens paper set in a home-made chamber. An ACSF at 30 - 32°C was run underneath a hippocampal slice at a flow rate of 0.3 ml min⁻¹. A 95% O₂/5% CO₂ gas mixture flowed over the slice. The slices were incubated for at least 30 min by running an ACSF. On the other hand, for a submerged slice preparation, a hippocampal slice was transferred on a lens paper in a chamber containing 0.50 ml of ACSF. A 95% O₂/5% CO₂ gas mixture flowed over the bath solution.

Preparation of a capillary sensor and current measurements
A glass capillary-based enzyme sensor was prepared as described in our previous paper. The inner solution was a Ca²⁺ and Mg²⁺-free ACSF (–3 μl) containing ascorbate oxidase (2 × 10⁸ U/ml). Amperometric measurements were performed at 0 V vs. Ag-AgCl with a computer-controlled electroanalytical system Cypress Model CS-1200 (Cypress Systems, KA). All of the measurements were carried out at room temperature. A home-made interface chamber or submerge chamber was set on a stage of a microscope equipped with a manipulator, NMN-25 (Narishige, Tokyo, Japan). Prior to monitoring L-glutamate release, a glass capillary electrode was operated in air at 0 V vs. Ag-AgCl until a steady current was obtained. In the case of interface preparation, the electrode was positioned on the surface of a target neuronal region, i.e. Cornu Ammonis 1 (CA1), followed by lowering it into the slice to a depth of ~10 μm with a manipulator. On the other hand, for a submerged slice, a glass capillary sensor was positioned once on the surface of region CA1 of a hippocampal slice, and then moved up into a bath solution 35 – 55 μm above CA1 with a manipulator.

Preparation of a patch sensor, current recordings and data analysis
A hippocampal slice was set in a superfusing chamber (0.50 mL) positioned under an upright microscope (BX50WI, Olympus, Tokyo, Japan). Superfusate (bath solution) was an ACSF. A patch sensor was prepared according to a procedure described in our previous paper. The composition of a pipet (inner) solution was the same as that of a bath solution. After a tight-seal (1 - 10 GΩ) out-side-out patch was formed by excising from the stratum pyramidale region of Cornu Ammonis 3 (CA3), the patch sensor was moved to approximately 25 μm above region CA1. Current recordings were performed at an applied potential of -60 mV with an Axopatch 200B patch-clamp amplifier with a built-in 1.0 kHz filter (Axon Instruments Inc., Burlingame, CA). Currents were stored on-line using a PHYSICOS PC computer (PYISICO-Tech Ltd., Tokyo, Japan) in which a pCLAMP software Ver. 6.04 or 8.0 (Axon Instruments Inc.) was installed. For calibrating the patch sensor, data acquisition with a sampling interval of 100 μs was continued for 20 s.

TEA stimulation
In interface slice preparation, approximately 10 min after the implantation of a glass capillary sensor, a small portion (0.20 mL) of a TEA solution was injected to the surface of a hippocampal slice with a micropipette. For a submerged slice, a 0.25-ml portion of a bath solution was taken out with a micropipette, and the same volume of a TEA solution was injected. This procedure was repeated three times to exchange the bath solution for a TEA solution. In the case of a patch sensor, TEA stimulation was performed by bath perfusion at a flow rate of approximately 0.4 mL/min.

Calibrating the sensor response of a capillary sensor
Calibrating the response of a glass capillary sensor in dip use was performed in the presence of a hippocampal slice. The sensor was positioned at a given height above CA1 and a standard L-glutamate solution was injected to give a known concentration (1.0 - 5.0 μM) of L-glutamate. The capillary sensor responded to L-glutamate in the concentration range from 1.0 to 5.0 μM (Fig. 1a). The concentration of L-glutamate released from a submerged hippocampal slice was evaluated based on the calibration curve.

Calibrating the response of an implanted capillary sensor was performed by injecting a small aliquot (5 μL) of a standard L-glutamate solution into region CA1 through the tip (~10 μm) of a glass capillary, which was implanted in the vicinity of the glutamate sensor. Pushing out an L-glutamate solution with gentle back pressure performed the injection. The obtained current vs. time traces is shown in Fig. 1b. The current reached a maximum, and then gradually decreased, seemingly due to the neuronal recovery of L-glutamate. A calibration curve for L-glutamate was prepared by plotting the maximum current against the L-glutamate concentration (Fig. 1c). It is noted that calibrating the sensor response at each neuronal region is necessary, because the activity of the L-glutamate uptake processes depends on each neuronal region.

Calibrating the response of a patch sensor
The response of a patch sensor was calibrated by injecting an L-glutamate solution to a bath solution (final concentration, 1.0 - 5.0 μM) and current recordings at ~60 mV were made for 20 s. Since the magnitude of the integrated current remained unchanged for 5 min, the response was evaluated at 1 min after L-glutamate injection. The response was defined as follows.

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Q(t) = Q(g) - Q(o),
\]
where \(Q(g)\) is the integrated current (coulomb) in a unit time (s) obtained in the presence of L-glutamate, and \(Q(o)\) is that in the absence of L-glutamate. A calibration curve for L-glutamate with a patch sensor is shown in Fig. 2. It is noted that the magnitude of the integrated current was smaller than reported in our previous paper, because co-agonist glycine was omitted from the bath and inner solutions, which is known to enhance the NMDA receptor currents.
Fig. 1 In situ calibration graphs for L-glutamate with a capillary sensor. The capillary sensors were operated at 0 V vs. Ag-AgCl. (a) A capillary sensor in dip use, (b) a current vs. time profile with an implanted sensor after the injection of L-glutamate, and (c) an implanted capillary sensor.

Fig. 2 Relationship between the integrated currents and the L-glutamate concentration with a patch sensor. The patch sensor was positioned 25 μm above CA1. A standard L-glutamate solution was injected to the bath solution. Current recordings were performed at −60 mV for 20 s.

Fig. 3 Current vs. time traces for 25 mM TEA-stimulated L-glutamate release with an implanted capillary sensor. The capillary sensors were implanted in region CA1 at a depth of approximately 10 μm. The operation potential was 0 V vs. Ag-AgCl. (a) Long-lasting case, (b) short-lasting case, and (c) control (ACSF). The black bar indicates the period where a TEA solution was injected and the grey bar the period where TEA stimulation was continued.

Result and Discussion

Monitoring of L-glutamate with a capillary sensor

The extracellular application of TEA is known to elicit chemically induced synaptic potentiation (cLTP) in CA1 of hippocampal slices.11-13 A TEA stimulation is known to activate both NMDA receptor channels and voltage-dependent calcium channels. The activation of the NMDA receptor channels induces calcium influx, often inducing LTP, which is similar to that evoked by a brief afferent tetanus (electrical stimulation). On the other hand, LTP induced by calcium channels is distinct from NMDA receptor-dependent LTP, and is based on a different type of synaptic modification.33 However, the nature and location, pre- or post-synaptic, of these modifications are still unknown. Knowing whether these potentiations of synaptic transmission enhance glutamate release will be interesting for understanding the fundamental potentiating processes. We reported that the application of TEA (25 mM) to the dentate gyrus (DG) region of an acute hippocampal slice induced an enhancement of L-glutamate release.15 In the present study, we
monitored L-glutamate release at CA1, because knowing the regional distribution of L-glutamate is important for understanding the fundamental neuronal processes. Figure 3 shows the amperometric monitoring of extracellular L-glutamate released from CA1 with a glass capillary sensor implanted at a depth of approximately 10 μm. A shift in the current appeared a few minutes after the application of TEA (traces a and b), while no changes in the current were observed when an ACSF was injected instead of TEA (trace c). Two cases of the TEA-induced responses were found, i.e., the current increased monotonously (trace a, n = 3) and the current returned to the initial level before TEA application (trace b, n = 3). Such profiles of TEA-stimulated glutamate release at CA1 were similar to those observed at DG.  

The instantaneous L-glutamate release suggests that the recovery processes of the neuronal system is active, which lowers the extracellular L-glutamate concentration. On the other hand, the long-lasting L-glutamate release implies that TEA-stimulated neuronal processes are active for an enhanced release of L-glutamate.

Monitoring L-glutamate release from CA1 with a glass capillary sensor in dip use is shown in Fig. 4. The sensors were positioned in a bath solution 40 μm (curve a) and 55 μm (curve b) above CA1, respectively. A shift in the reduction current was induced a few minutes after the exchange of a bath solution for a TEA solution. The current vs. time profiles were essentially similar to those observed with an implanted capillary sensor (vide supra). This suggests that the diffusion of L-glutamate from the slice interior into a bath solution inevitably occurs, and the extra-slice level of L-glutamate reflects a change in the extracellular one.

Fig. 4 Current vs. time traces for 25 mM TEA-stimulated L-glutamate release with a capillary sensor in dip use. The sensor was positioned above CA1 in a bath solution. The operation potential was 0 V vs. Ag-AgCl. (a) Long-lasting case and (b) short-lasting case. The black bar indicates the period where a TEA solution was injected and the grey bar the period where TEA stimulation was continued.

Fig. 5 Current vs. time traces for 25 mM TEA-stimulated L-glutamate release with a patch sensor positioned 25 μm above CA1. The operation potential was −60 mV vs. Ag-AgCl. At the initial stage of the recording, the applied voltage was switched to 0 mV to confirm a stable patch membrane. (a) Long-lasting case, (b) short-lasting case, and (c) control (ACSF).

Monitoring of L-glutamate with a patch sensor
Current traces obtained by the perfusion of a TEA solution are shown in Fig. 5. Approximately 3 min after TEA perfusion, channel currents appeared, indicating the release of L-glutamate, and the response continued for several minutes. We noticed two cases of the current-time profiles. First, the induction of a channel current continued even after the perfusion of an ACSF (trace a, n = 6). Second, the TEA-induced currents were abolished when an ACSF was perfused (trace b, n = 4). The perfusion of an ACSF caused no noticeable channel currents until 20 min (trace c, n = 5). When a patch sensor was positioned out of a hippocampal slice and a TEA solution was perfused, the sensor exhibited no responses, indicating that the response observed as mentioned above is ascribable to L-glutamate released from region CA1 of the submerged slice.

To correlate the observed channel currents to the L-glutamate concentration, the current traces were integrated every 1 s after TEA application. The integration was performed for a period of 20 s, because the calibration of a patch sensor was based on a recording period of 20 s (vide supra). The responses were analyzed for 13 – 15 min after TEA stimulation, because at a longer recording time the patch sensor often exhibited large background currents caused by a leaky membrane. Time-divided integrated current vs. time plots are shown in Fig. 6. The response profiles were very similar to those observed with a capillary sensor in dip use in the sense that two cases, i.e., long-lasting (trace a) and short-lasting (trace b) cases, were observed. The extra-slice concentration of L-glutamate was evaluated from the average of the integrated currents for between 3 and 5 min, corresponding to the time interval required to attain the maximum current with a capillary sensor.
Comparison of L-glutamate levels at CA1

A comparison of the L-glutamate level between two types of slice preparations is given in Table 1. In submerged slice preparation, the concentration level of L-glutamate at CA1, obtained with the capillary sensor, was very close to that obtained with the patch sensor. The agreement of the extra-slice concentration between the two sensors demonstrates that one can accurately measure the extra-slice level of L-glutamate by the respective methods. On the other hand, the concentration of L-glutamate obtained with the implanted capillary sensor was significantly larger than those obtained with the capillary sensor in dip use. This is simply because the implanted sensor measures extracellular L-glutamate before it diffuses away from the neuronal sites. The extracellular L-glutamate level at CA1 under TEA stimulation was in the range from 6 to 10 μM, which was slightly larger than the basal level (approximately 1.8 μM), but smaller than those at DG. The lower L-glutamate level at CA1 suggests that L-glutamate in CA1 is more intensely removed from the extracellular space, possibly because the uptake process is more active. This explanation is supported by an observation that a decrease in the glutamate current after the injection of L-glutamate at CA1 (Fig. 1b) was much larger than that at DG. In addition, the order of L-glutamate release is in accordance with a result obtained by the imaging method in the sense that the uptake of L-glutamate at CA1 is highly sensitive to an excitatory amino acid uptake inhibitor, DL-TBOA.

Conclusion

The results mentioned above demonstrate that one can accurately monitor TEA-stimulated L-glutamate release from acute hippocampal slices with a capillary-based enzyme sensor and a patch sensor. Although the extra-slice L-glutamate concentration is lower than the intra-slice (extracellular) one, the extra-slice concentration is indicative of the extracellular one. The extracellular level of L-glutamate at CA1 under TEA stimulation was several μM, which is lower than that at DG, reflecting the activity of neuronal uptake processes. The enzyme and patch sensors can purposely be used for monitoring L-glutamate release in different preparations of acute hippocampal slices.

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References


Table 1 Comparison of intra-slice and extra-slice concentrations of L-glutamate released from regions CA1 and DG of mouse hippocampal slices by TEA (25 mM) stimulation

<table>
<thead>
<tr>
<th>Type of slices</th>
<th>Sensor</th>
<th>Response type</th>
<th>L-Glutamate concentration/μM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interface</td>
<td>Implanted CEa</td>
<td>Long-lasting</td>
<td>6 ± 4 (n = 3)t</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Short-lasting</td>
<td>10 ± 2 (n = 3)t</td>
</tr>
<tr>
<td>Submerge</td>
<td>CEa</td>
<td>Long-lasting</td>
<td>1.0 ± 0.1 (n = 3)t</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Short-lasting</td>
<td>2.3 ± 1.0 (n = 6)t</td>
</tr>
<tr>
<td>Submerge</td>
<td>Patch sensorc</td>
<td>Long-lasting</td>
<td>2.0 ± 1.5 (n = 6)t</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Short-lasting</td>
<td>4.0 ± 1.6 (n = 4)t</td>
</tr>
</tbody>
</table>

a. A capillary sensor implanted in the slice at a depth of approximately 10 μm from the surface of the target region. b. A capillary sensor positioned in a bath solution (ACSF) 35 – 55 μm above the target region. c. A patch sensor positioned in a bath solution (ACSF) approximately 25 μm above the target region. d. L-Glutamate concentration obtained from the maximum current. e. L-Glutamate concentration obtained from the averaged integrated currents between 3 and 5 min after TEA stimulation. f. Data from Ref. 15.