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Long-Term Potentiation at Hippocampal Perforant Path-Dentate Astrocyte Synapses

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Abstract

Accumulated evidence indicates that astroglial cells actively participate in neuronal synaptic transmission and plasticity. However, it is still not clear whether astrocytes are able to undergo plasticity in response to synaptic inputs. Here we demonstrate that a long-term potentiation (LTP)-like response could be detected at perforant path-dentate astrocyte synapses following high-frequency stimulation (HFS) in hippocampal slices of GFAP-GFP transgenic mice. The potentiation was not dependent on the glutamate transporters nor the group I metabotropic glutamate receptors. However, the induction of LTP requires activation of the NMDA receptor (NMDAR). The presence of functional NMDAR was supported by isolating the NMDAR-gated current and by identifying mRNAs of NMDAR subunits in astrocytes. Our results suggest that astrocytes in the hippocampal dentate gyrus are able to undergo plasticity in response presynaptic inputs.

Growing evidence suggests that astrocytes, enveloping synapses and forming tripartite synapses [1,2], actively participate in synaptic transmission and plasticity in the brain [3,4]. It has been demonstrated that elevated cytoplasmic Ca^{2+} in response to presynaptic released neurotransmitters leads to the release of several neurotransmitters and modulators (e.g., glutamate, ATP, prostaglandin, and D-serine) from astrocytes [5–8]. These neuroactive factors act, in turn, on neuronal pre- and postsynaptic sites to regulate the efficacy and strength of synapses [1,8,9]. Importantly, the presence of ligand-gated ionotropic receptor channels, such as AMPA and glutamate transporters, including glutamate-aspartate transporter (GLAST) and glutamate transporter 1 (GLT-1), enables astrocytes to directly display electrical responses to neurotransmitters resulting from neuronal activity.

Long-term potentiation (LTP) is a form of synaptic plasticity evoked by high-frequency stimulation (HFS) and is believed to be a neuron-specific response [10]. While neuronal LTP has been extensively investigated in distinct synapses and various regions, our understanding of the role of astrocytes in neuronal LTP is still primitive. It has been shown previously that an LTP-like response was detected in granule neuron-glial cell pairs in cerebellar cultures [11,12], indicating that astrocytes are able to undertake behavioral changes in response to synaptic inputs. A recent report shows LTP in NG2 cells, a precursor cell of oligodendrocytes [13], meaning that glial cells are able to undergo plasticity in response to synaptic inputs [16]. However, little information is available on whether synaptic plasticity can be induced at perforant path-astrocyte synapses. This is largely due to the fact that most astrocytes are located predominantly in the granule cell body layer, and are difficult to distinguish from neurons [14]. The use of GFAP-GFP transgenic mice allows us to identify and visualize astrocytes with GFP fluorescence, and thus to characterize their biophysical properties and responses to synaptic inputs. Here we report that an LTP-like response can be induced by HFS in astrocytes

in the hippocampal dentate gyrus. The potentiation is independent on glutamate transporter currents and mGluRs, but requires activation of the NMDA receptor. Our results suggest that astrocytes behave like neurons undergoing plasticity in response to LTP stimulation.

Materials and methods

Hippocampal slice preparation

Hippocampal slices were prepared from 5- to 12-week-old GFAP-GFP transgenic FVB/N mice (Jackson Laboratory, Bar Harbor, ME) of either sex as previously described [15,16] using a protocol approved by the Institutional Animal Care and Use Committee of the Louisiana State University Health Sciences Center. Briefly, slices were cut at a thickness of 400 μm in a cold oxygenated (95% O_2 -5% CO_2) low- Ca^{2+} /high- Mg^{2+} slicing solution composed of (in mM) 2.5 KCl, 7.0 MgCl_2 , 28.0 NaHCO_3 , 1.25 NaH_2PO_4 , 0.5 CaCl_2 , 7.0 glucose, 3 pyruvic acid, 1 ascorbic acid, and 234 sucrose. Then, the slices were transferred to a holding chamber containing oxygenated ACSF composed of (in mM) 125.0 NaCl, 2.5 KCl, 1.0 MgCl_2 , 25.0 NaHCO_3 , 1.25 NaH_2PO_4 , 2.0 CaCl_2 , 25.0 glucose.

Electrophysiological recordings

Astrocytes were identified by GFP fluorescence under ultraviolet illumination. Whole-cell current clamp (AxoClamp 2B) or voltage clamp (Axopatch 200A) recordings were made in astrocytes located at the granule cell body layer in the dentate gyrus. The electrodes (3–4 M Ω) were filled with the internal solution containing (mM) 120 K gluconate, 20 KCl, 4 NaCl, 0.28 CaCl_2 , 0.5 EGTA, 10 HEPES, 4 MgATP, 0.3 Tris-GTP, and 14 Na_2 Phosphocreatine, pH was adjusted to 7.3 with KOH. To increase the size of the glutamate transporter currents, gluconate was substituted by methanesulfonate in the internal solution as described previously [17,18]. Excitatory postsynaptic astrocytic potentials (EPAPs) or currents (EPACs) were elicited by delivering a stimulus of 200 μs in duration at a frequency of 0.05 Hz via a bipolar tungsten electrode placed at the molecular layer (perforant path). LTP was induced by a high-frequency stimulation protocol, consisting of 8 trains, each of 8 pulses at 200 Hz with an inter-train interval of 2 seconds, as described previously [15,16]. To eliminate Mg^{2+} -induced blockade of the NMDA receptors, the slices were incubated in Mg^{2+} -free solution containing 1 mM kynurenic acid (Kyn) for at least 2 hours, then perfused with the normal Mg^{2+} -free solution for at least 40 min to wash out Kyn before recordings. The bath solution contained 10 μM bicuculline to block ionotropic GABA receptors.

Single-cell reverse transcription-PCR (RT-PCR)

Single-cell RT-PCR was performed as we previously described [19]. Briefly, whole-cell patch clamp was made in astrocytes. The cytoplasm of the cells was harvested by gentle suction into the patch pipettes filled with (mM) 140 KCl, 5 HEPES, 5 EGTA, 3 MgCl_2 ; pH was adjusted to 7.3. The pipette contents were rapidly expelled into a tube containing 5 μM random hexamer primers (Boehringer Mannheim), 0.5 mM each of the four deoxyribonucleotide triphosphates (Pharmacia), 10 mM dithiothreitol, 20 U ribonuclease inhibitor (Promega), and 100 U reverse transcriptase (Invitrogen). First-strand single-cell cDNA was synthesized for 10 min at 25 $^{\circ}\text{C}$, 1 hr at 42 $^{\circ}\text{C}$, and 5 min at 70 $^{\circ}\text{C}$ in a total reaction volume of 10 μl . After reverse transcription, two rounds of PCR were performed as described [20]. The cDNAs for NR1, NR2A, NR2B, and NR2C were amplified simultaneously using the primers (supplemental Table 1). All individual PCR products were gel purified by Qiagen kit and confirmed by sequencing. Total RNA was prepared from mouse hippocampal slices using Absolutely RNA Kit (Stratagene). The reverse transcription was performed with 500 pg of total RNA as the positive control for each single-cell amplification.

Data were presented as mean $\pm$ SEM. Student's t-test and analysis of variance (ANOVA) with Student-Newman-Keuls test were used for statistical comparison when appropriate. Differences were considered significant when $P < 0.05$.

Results

LTP induction at perforant path-granule neuron-astrocyte synapses

Astrocytes located at the granule cell body layer in the dentate gyrus were identified by visualized green fluorescence under ultraviolet excitation illumination in acutely prepared hippocampal slices. They showed relatively small and thin soma under differential interference contrast (DIC) optics. Electrophysiological recordings showed that the resting membrane potential (-86.4 ± 0.2 mV, $n = 107$ cells) in these cells was slightly more negative than that in granule neurons (-83.2 ± 0.4 mV, $n = 28$ cells, $P < 0.01$). Striking characteristics of these cells were no firing upon depolarizing current injections (Fig. 1A), and lower input resistance (9 ± 1 M Ω , $n = 44$ cells) when compared to that of granule neurons (236 ± 8 M Ω , $n = 24$, $P < 0.01$, Fig. 1B). Moreover, the EPAP had relatively small amplitude and slow kinetics (10–90% rise time: 10.93 ± 1.04 ms, 10–90% delay time: >1000 ms, $n=16$).

HFS-induced LTP induction at perforant path has been well established in our lab [15,16]. To test whether a similar paradigm could elicit an LTP-like event in astrocytes in response to HFS at the perforant path, EPAPs were recorded in astrocytes under whole-cell current clamp configuration. EPAPs were significantly potentiated following HFS and lasted for more than 30 minutes (Fig. 1C). The magnitude of the EPAP enhancement was more pronounced when compared to that of EPSPs during neuronal LTP induced by HFS at the perforant path (EPAPs: $428.1 \pm 49.6\%$ of baseline during the first 1–5 min following HFS, and $325.8 \pm 24.1\%$ of baseline during 25–30 min after HFS, $n=14$, vs. EPSPs: 278.5 ± 27.6 and $193.2 \pm 21.1\%$ of baseline, respectively, $n=12$, $P < 0.01$; Fig. 1C). These results indicate that an LTP-like response can be induced following HFS in hippocampal dentate gyrus astrocytes.

Glutamate transporters do not contribute to astrocytic LTP

Astrocytes express two primary functional glutamate transporters, GLAST and GLT-1 [21]. As demonstrated previously, the induction of LTP at perforant path is accompanied by increased presynaptic release of glutamate [22]. To determine whether glutamate transporter currents during neuronal LTP contribute to the enhanced EPAPs, THA (150 μ M), a GLAST inhibitor, and DHK (150 μ M), a GLT-1 inhibitor, was applied to slices. These inhibitors slightly reduced the membrane potential by 3.5 ± 0.4 mV ($n=11$), and caused a transient increase (lasting for about 2 to 3 min) and subsequent decrease in EPAPs (Fig. 2A). The transporter inhibitor-induced initial increase in EPAPs is likely due to the inhibition of glutamate transporters that cause a rapid glutamate accumulation at the synaptic cleft, which induces an enhanced glutamate receptor-mediated response, whereas the subsequent decrease in EPAPs likely results from a desensitization of AMPA receptors [23] or an activation of presynaptic metabotropic glutamate receptors that inhibit presynaptic release of glutamate [24]. It appeared that EPAPs were still enhanced following HFS in the presence of transporter inhibitors ($338.5 \pm 39.0\%$ of baseline, at the first 1–5 min after HFS, and $320.1 \pm 31.2\%$ of baseline at 26–30 min after HFS, $n=6$; Fig. 2A). There were no significant differences in the magnitudes of the EPAPs after HFS in the presence of transporter inhibitors when compared to that in control ($P > 0.05$). In addition, a short period of application of DHK (300 μ M) alone (Fig. 2B), or together with THA (300 μ M), did not affect LTP induction ($365.5 \pm 38.2\%$ of baseline at 26–30 min after HFS, $n=6$; $362.0 \pm 30.6\%$ of baseline at 16–20 min after HFS, $n=7$, respectively). These results suggest that glutamate transporters did not significantly contribute to astrocytic LTP.

To determine the contributions of glutamate transporter-mediated currents to EPAPs, we recorded synaptically evoked excitatory postsynaptic astrocytic currents (EPACs) under voltage clamp mode in the presence of the non-selective ionotropic glutamate receptor antagonist Kyn (1.5 mM). The inhibition of the glutamate receptor-mediated currents resulted in reduction of the remaining currents to $11.3 \pm 1.9\%$ ($n=6$) and $6.7 \pm 0.6\%$ of the baseline before and after HFS, respectively (Fig. 2C). The residual currents were 13.7 ± 2.1 pA and 20.8 ± 1.9 pA before and after induction of LTP, respectively ($P<0.05$). Presumably, these were the transporter currents (Fig. 2D). These results indicate that the transporter currents only occupied a small proportion of EPAPs, especially during LTP, suggesting that EPAPs are mainly composed of ionotropic glutamate receptor-gated current components, especially after induction of LTP. This is in harmony with the results reported by others [11,12]. Although there was only a small increase in the amplitude of glutamate transporter currents following HFS, this indicates that the presynaptic release of glutamate is enhanced during LTP at the hippocampal perforant path. This means that astrocytic LTP at the perforant path is mainly presynaptically expressed, similar to neuronal LTP at perforant path-dentate granule cell synapses [22].

Metabotropic glutamate receptors are not involved in astrocytic LTP

Several lines of evidence indicate that metabotropic glutamate receptors (mGluR) are expressed in astrocytes [25,26]. Group I mGluRs are coupled to phospholipase C and stimulate inositol phosphate metabolism and mobilization of intracellular Ca^{2+} , whereas Group II and III are coupled to adenylyl cyclase and down-regulate cAMP formation. Since a rise in intracellular Ca^{2+} is critical for neuronal LTP induction, we decided to examine the role of mGluR_{1/5} in astrocytic LTP. As indicated in figure 3A, bath application of DHPG (30 μM), a group I mGluR agonist, did not significantly change the magnitudes of EPAPs ($435 \pm 50\%$ and $405 \pm 54\%$ of the baseline at 1–5 and 26–30 min after HFS, respectively; $n=11$). Application of AIDA (500 μM), a group I mGluR antagonist, also did not significantly alter LTP ($476 \pm 71\%$ and $355 \pm 23\%$ of the baseline at 1–5 and 26–30 min following HFS, respectively; $n=4$, figure 3B). We also used CHPG (500 μM), another group I mGluR agonist, and we found that there were no significant differences in the magnitudes of EPAPs between the control and CHPG ($320.1 \pm 31.2\%$, $n=6$; versus $314 \pm 61\%$ of baseline at 26–30 min, $n=4$; $P>0.05$). These data suggest that group I mGluRs may not be involved in HFS-induced LTP in astrocytes.

LTP induction in astrocytes requires activation of the NMDA receptor

Since the majority of EPAP components resulted from the glutamate receptor-mediated currents, we decided then to determine whether the ionotropic glutamate receptors are responsible for LTP induction in astrocytes. DNQX (10 μM), or APV (50 μM), or Kyn (1.5 mM) was applied after stable EPAPs were acquired for 5 minutes. Application of these receptor antagonists resulted in decreases in EPAPs by $82.3 \pm 3.4\%$ (DNQX, $n=9$), $35.6 \pm 2.7\%$ (APV, $n=9$) and $88.8 \pm 3.0\%$ (Kyn, $n=6$), respectively (Figure 3C). EPAPs were then gradually enhanced to $280.5 \pm 31.6\%$ of baseline ($n=9$) after washout of DNQX for 25 min following HFS. However, the HFS-induced enhancement of EPAPs was completely inhibited (APV: $108.1 \pm 11.5\%$ of baseline, $n=9$; and Kyn: $111.3 \pm 10.3\%$ of baseline, $n=6$) upon washout of APV or Kyn (Figure 3C). Similar effects were observed when NBQX or MK-801 was applied (data not shown). These results indicate that activation of the NMDA receptor is required for LTP induction in astrocytes, and the AMPA receptor-mediated currents are the major components in EPAPs.

Functional NMDA receptors are expressed in astrocytes

It has been generally agreed that AMPA receptors are expressed in glial astrocytes [27]. However, it was still uncertain whether the NMDA receptor is expressed in astrocytes [28,

29]. Since LTP in astrocytes is blocked by NMDA receptor antagonists, but not by AMPA receptor antagonists, this implies that there might be functional NMDA receptors expressed in astrocytes. To test this hypothesis, the NMDA receptor-mediated current was isolated in astrocytes under a whole-cell voltage clamp configuration in an Mg^{2+} -free external solution. Synaptically evoked inward currents were induced in response to stimuli at the perforant path at a holding potential of -85 mV. Application of NBQX ($20 \mu M$) reduced the total currents to $47.7 \pm 6.9\%$ of the baseline ($n=9$). Subsequently, THA ($300 \mu M$) and DHK ($300 \mu M$) were applied simultaneously. The glutamate transporter inhibitors reduced the total current slightly, to $40.4 \pm 7.1\%$. Interestingly, the remaining current was completely eliminated by $50 \mu M$ APV (Figure 4A). This result implies that the NMDA receptor is likely expressed on the membranes of astrocytes [29], and functionally contributes to synaptically evoked EPAPs and LTP in astrocytes.

To further confirm the expression of the NMDA receptor in astrocytes, single-cellular mRNAs were harvested and isolated from astrocytes using a single-cell RT-PCR technique as we previously described [19]. As shown in figure 4B, significant amplification signals for NMDA receptor subunits NR1, NR2A, and NR2B were detected. These data together with the NMDA receptor-mediated current suggest that the NMDA receptor is functionally expressed in astrocytes [29].

Discussion

LTP in the mammalian hippocampus has been commonly regarded as a neuron-specific phenomenon. In the present study, we demonstrated that an LTP-like event in response to HFS at the perforant path could be detected in dentate gyrus astrocytes in hippocampal slices. This enhanced astrocytic response following HFS appears to result from the increased presynaptic release of glutamate. These results indicate that LTP can be induced in dentate gyrus astrocytes *ex vivo*, which is in harmony with the observation made in granule neuron-astrocyte pairs in cerebellar cultures [11,12], suggesting that astrocytes are able to change their behaviors in response to synaptic inputs.

The activation of AMPA receptors allows ion influx into astrocytes through the receptor channels, while activity of glutamate transporters produces a net inward current by the uptake of the released glutamate along with co-transported $3Na^+$ and $1H^+$, and counter-transported $1K^+$ [21]. This indicates that changes in the membrane potential of astrocytes result not simply from redistribution of extracellular potassium, but also from the cellular response to synaptic activity [30]. An important role of astrocytes is to clean up the released glutamate during synaptic activity through glutamate transporters. Activation of the glutamate transporters produces net inward currents that depolarize the membrane potential of astrocytes. Tetanic stimulation of cerebellar neurons has been shown to induce LTP of glutamate transporter currents in associated astrocytes or in postsynaptic neurons [12,31]. It is likely that the enhanced transporter currents after HFS contribute to astrocytic LTP, which results from the increased presynaptic release of glutamate. However, there are no changes in the glutamate transporter currents in astrocytes during neuronal LTP in the hippocampal CA1 area [18]. In the present study, we observed that there is an increase in the transporter currents after LTP. This indicates that the presynaptic release of glutamate is increased after HFS. Since the glutamate transporter current contributes a small percentage ($\sim 10\%$) to the total synaptically evoked EPACs, especially during LTP ($\sim 6\%$), it is unlikely that the glutamate transporter currents contribute greatly to LTP in astrocytes. Also application of group I mGluR agonists or antagonist did not affect HFS-induced LTP induction in astrocytes, suggesting that mGluRs may not participate in astrocytic LTP.

In the present study, we demonstrated that activation of the NMDAR is required for LTP induction in astrocytes. This is supported by the evidence that LTP is inhibited by NMDAR blockers and functional NMDARs are expressed in astrocytes in terms of identification of mRNAs of the NMDAR subunits and isolation of the NMDAR-gated current. This means that induction of LTP in astrocytes is similar to that in neurons. The elevated astrocytic responses to presynaptic inputs may play an important role in neuronal synaptic transmission and plasticity in the hippocampus.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Biophysical properties of astrocytes and LTP induction at the hippocampal perforant path. A1-2. Representative membrane potential response to hyperpolarizing and depolarizing current injections (250 ms duration from -150 to 250 pA) in an astrocyte and a granule neuron in the hippocampal dentate gyrus. B. Input resistance between astrocytes and granule neurons measured with voltage deflections in response to current injections. C1. Representative traces before HFS (1) and at 30 min after HFS (2) from an astrocyte and a neuron. C2. Synaptically evoked EPAPs and neuronal EPSPs were potentiated following HFS (arrow).

Figure 2.

Glutamate transporter-mediated currents do not contribute to astrocytic LTP.

A1. Representative traces in the absence (1), 2-min (2), or 5-min (3) after application of glutamate transporter inhibitors THA (150 μ M) and DHK (150 μ M), and at 30 min after HFS (4). A2. The time course of EPAPs. B1 Representative traces in the absence (1), 5 min (2) application of 300 μ M DHK, and at 30 min after HFS (3). B2. The time course of EPAPs. C1. Representative synaptically evoked excitatory postsynaptic astrocytic currents (EPAC) before and after application of HFS. C2. The time courses of EPSC inhibition by ionotropic glutamate antagonist. D. Mean values of EPACs during application of Kyn (1.5 mM) before and 10 min after HFS. * $P < 0.05$.

Figure 3.

Astrocytic LTP requires activation of the NMDA receptor. A1. Representative traces in the presence of DHPG before and after HFS. A2. The time course of astrocytic LTP in the presence of DHPG (30 μ M). B1. Representative traces in the presence of AIDA before and after HFS. B2. The time course of astrocytic LTP in the presence of AIDA (500 μ M). C1. Representative traces in the presence DNQX, or APV, or Kyn, and at 30 min after HFS. C2. Time courses and extent of LTP following HFS. C3. Mean values of EPAPs at baseline, 1–5 min and 26–30 min after HFS in the presence of glutamate receptor antagonists. ** $P < 0.01$ vs. control.

Figure 4. Functional NMDA receptors are expressed in astrocytes

A1. Synaptically evoked current components were isolated by sequential applications of vehicle (1), NBQX (2), THA+DHK (3), and APV (4) in the Mg^{2+} -free external solution. A2. Time course of changes in EPACs in response to different treatments. B. The mRNA of NMDA receptor subunits NR1, NR2A, NR2B, and NR2C were identified using single-cell RT-PCR analysis. RT-PCR products from astrocytes (n=2) are showed in lane 2; RT-PCR products from positive control (RNA isolated from mouse hippocampal tissue) and negative control (the pipette solution) are showed in lanes 1 and 3, respectively.