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Synapse-specific effects of IL-1β on long-term potentiation in the mouse hippocampus

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ABSTRACT

Interleukin-1β (IL-1β) is a key molecule in the inflammatory responses elicited during infection and injury. It exerts local effects on synaptic plasticity by binding to IL-1 receptors that are expressed at high levels in the hippocampus. We examined the effects of IL-1β on synaptic plasticity in different hippocampal regions in acute mouse brain slices by measuring long-term potentiation (LTP). IL-1β (1 ng/mL) was applied for 30 min before LTP was induced with high-frequency stimulation (HFS). LTP was significantly impaired by either IL-1β application to the Schaffer collateral-CA1 synapses or the associational/commissural (A/C) fiber-CA3 synapses, which are both dependent on N-methyl-D-aspartate (NMDA) receptor activation. However, mossy fiber-CA3 LTP, which is expressed presynaptically in an NMDA-independent manner, was not impaired by IL-1β. Our results demonstrate that IL-1β exerts variable effects on LTP at different kinds of synapses, indicating that IL-1β has synapse-specific effects on hippocampal synaptic plasticity.

The immune system and brain constantly communicate with each other in both sickness and health. Systemic inflammation due to sepsis and surgery can disrupt the blood-brain barrier (16, 24), and elevated levels of pro-inflammatory cytokines in the brain have pathological effects on neural function (15, 22). It is well known that pro-inflammatory cytokines modulate synaptic plasticity (15, 22) including long-term potentiation (LTP), which is considered the cellular basis of learning and memory (2, 18). Interleukin-1β (IL-1β) is a key molecule involved in inflammatory responses to infection and injury that is released from microglia in the brain; it exerts local effects on hippocampal synaptic plasticity through unknown mechanisms at IL-1 receptors, which are expressed at high levels in the hippocampus (8, 21). To reveal the effects of IL-1β on hippocampal synaptic plasticity, the acute hippocampal slice method has been used in most of the previous studies (1, 5, 7, 11, 17); it is regarded as the gold standard model for analysis of neural function due to its viability of synaptic circuits (28). Although several studies have reported that IL-1β impairs LTP in the Schaffer collateral (SC)-CA1 (1) and perforant path-dentate gyrus pathways (5, 7, 17), only one study has examined its effects in the CA3 region (11). Two types of LTP occur in the CA3 region. The first is associational/commissural (A/C) fiber LTP, which is dependent on N-methyl-D-aspartate (NMDA) receptor activation similar to LTP in other hippocampal synapses. The other is mossy fiber LTP, which is independent of NMDA receptor activation and is expressed presynaptically (19). Although Katsuki *et al.* reported that IL-1β impaired LTP at mossy fiber-CA3 synapses in acute mouse hippocampal slices, they did not use any methods to consider excitatory postsynaptic potentials (EPSPs) as pure mossy fiber signals, which made it difficult to discriminate between the two pathways (mossy fiber and A/C signals) (19). Therefore, we examined the effects of IL-1β on mossy fiber LTP and A/C fiber LTP in the CA3 region with

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confirmation methods of mossy-fiber signals in addition to SC-CA1 LTP in acute hippocampal slices obtained from mice.

MATERIALS AND METHODS

Animals and slice preparations. All experiments were performed according to the guidelines for the care and use of laboratory animals of Hokkaido University. Male and female *C57BL/6J* mice were used in the present study. Transverse hippocampal slices (300 μm thick) were prepared from mice (4–6 weeks old). Animals were anesthetized with ether, and the brain was dissected in an ice-cold sucrose solution composed of the following (in mM): 40 NaCl, 25 $NaHCO_3$, 10 glucose, 150 sucrose, 4 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, and 7 $MgCl_2$. Transverse slices were cut with a DTK-1000® microslicer (DOSAKA-EM, Kyoto, Japan). Then, the sucrose-containing solution was replaced with artificial cerebrospinal fluid (ACSF) containing the following (in mM): 127 NaCl, 1.5 KCl, 1.2 KH_2PO_4, 26 $NaHCO_3$, 10 glucose, 2.4 $CaCl_2$, and 1.3 $MgCl_2$, and the slices were incubated for 30 min at 30°C and subsequently for approximately 30 min at room temperature in an interface-type chamber with saturated 95% O_2 and 5% CO_2.

Electrophysiology. Slices were transferred into an observation chamber and continuously superfused at 2 mL/min with ACSF that was equilibrated with 95% O_2 and 5% CO_2. For SC-CA1 synapse experiments, electrical stimuli (200 μs duration, <300 μA intensity) were delivered every 10 s through a tungsten concentric bipolar electrode inserted into the stratum radiatum of the CA1 region, and the resultant EPSPs were recorded from the same region via glass microelectrodes with 10-μm diameter tips filled with ACSF. Stimulation intensity was increased up to the subthreshold causing action potentials to induce maximum LTP. For experiments at A/C fiber synapses, stimulating and recording electrodes were placed in the stratum radiatum of the CA3 region, and the same electrical stimuli were delivered as for the CA1 experiments. In experiments at mossy fiber-CA3 synapses, the granule cell layer of the dentate gyrus was stimulated, and the evoked EPSPs were recorded in the stratum lucidum of the CA3 region. Mossy fiber synapses onto CA3 pyramidal cells are distinct from most other synapses because of their large paired-pulse facilitation, with a ratio around 3 in most cases (26). Therefore, paired-pulse stimuli (200 μs duration, <900 μA intensity) with a 50-ms interval were applied for recordings at mossy fiber-CA3 synapses, and we considered EPSPs with paired-pulse ratios ($EPSP_2/EPSP_1$) >2.5 as mossy fiber signals.

Furthermore, we used (2S,2'R,3'R)-2-(2',3'-dicarboxycyclo-propyl) glycine (DCG-IV, 1 μM: Tocris Bioscience, Ellisville, MO, USA) at the end of experiments to confirm that mossy fibers were selectively stimulated (10), and the recordings only with significant reductions (≤80%) of EPSPs from the baseline were included in the statistical analysis. High-frequency stimulation (HFS) was used to induce LTP. Three trains of 100-Hz stimulation for 1 s (0.05 Hz) were applied for SC-CA1 and A/C fiber-CA3 synapses to induce maximum LTP, and a train of 100-Hz stimulation for 1 s was used for the mossy fiber-CA3 synapses because our preliminary experiments showed no difference between the amplitudes of potentiated EPSPs at mossy fiber-CA3 synapses regardless of the number of train stimulations (data not shown). For LTP experiments at mossy fiber synapses, 25 μM of the selective NMDA receptor antagonist D-AP5 (Tocris Bioscience) was applied 10 min before HFS to prevent contamination of NMDA receptor-dependent potentiation at the CA3 recurrent collaterals. All recordings were made at room temperature (24–26°C) using a MultiClamp® 700B amplifier (Molecular Devices, Sunnyvale, CA, USA) and pClamp® 10.0 software (Molecular Devices).

Drugs. Mouse recombinant IL-1β was obtained from Sigma-Aldrich (St. Louis, MO, USA) with 98% purity (the endotoxin level ≤0.1 ng/μg). Bovine serum albumin (BSA, 1 μg/mL) was used as a carrier protein to prevent nonspecific absorption of IL-1β to the recording chamber, tubing, etc. While the drug was delivered to slices, solution containing IL-1β (1 ng/mL) was kept in a polypropylene container for the same reason. For control group recordings, BSA (1 μg/mL) was applied instead of IL-1β. The stored IL-1β solution was used after only one freeze-thaw cycle to prevent its inactivation.

Statistics. The statistical significances of LTP values were analyzed using Student's *t* tests after confirming that data were normally distributed (Shapiro-Wilk tests). We used Mann-Whitney U tests unless they were normally distributed. Statistical analysis was performed with JMP® Pro 11.0 (SAS Institute Inc., Cary, NC, USA). Experimental values are expressed as the mean ± standard error of the mean (SEM). Values were considered statistically significant at $P < 0.05$.

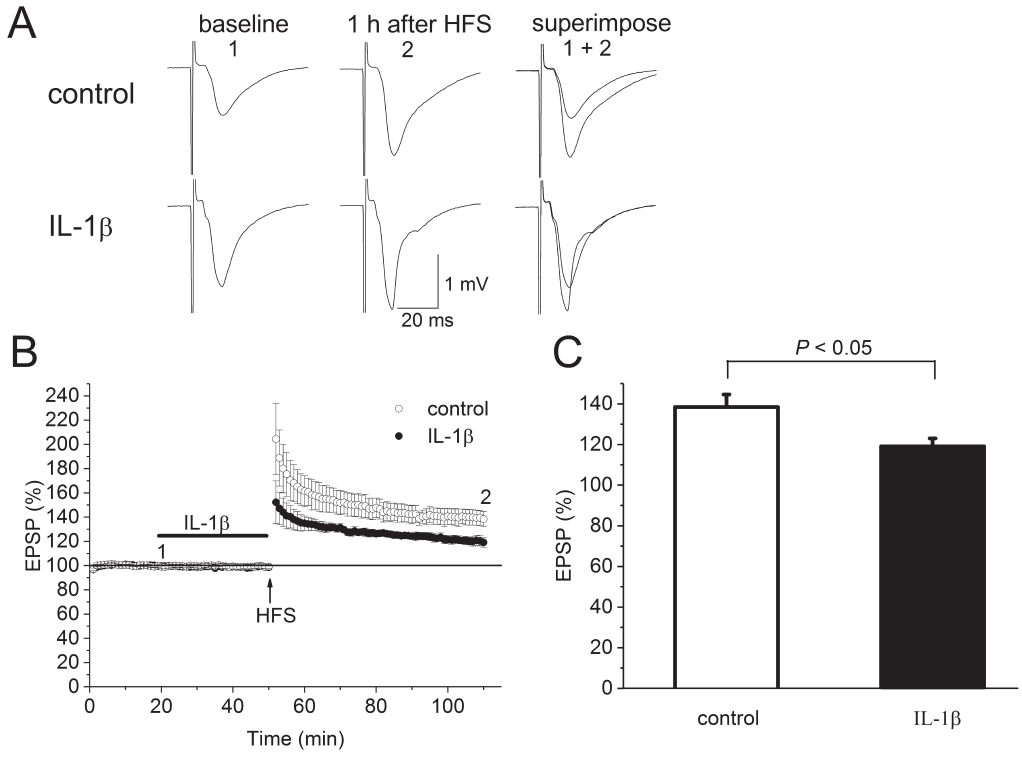

Fig. 1 IL-1β impairs Schaffer collateral-CA1 LTP. (**A**) Effect of IL-1β application (1 ng/mL) on LTP at CA1 synapses. (**B**) Time course of EPSP amplitudes in the above experiment. LTP was induced with three trains of 100 Hz for 1 s repeated at 0.05 Hz. Representative examples in A were sampled at the time points labeled by the numbers. (**C**) Percentage of mean EPSP amplitude 60 min after LTP induction against baseline in the control and IL-1β groups. Data are expressed as mean ± SEM. HFS: high-frequency stimulation.

RESULTS

We first examined the effects of IL-1β on NMDA receptor-dependent LTP at SC-CA1 synapses. When three trains of 100 Hz for 1 s (0.05 Hz) were delivered to slices in control group, EPSPs immediately increased, resulting in robust LTP (138.4 ± 6.2% of EPSP amplitude 60 min after HFS compared to baseline, $n = 4$). However, 30-min IL-1β (1 ng/mL) administration 20 min after recordings stabilized the baseline of EPSP amplitude, significantly impaired LTP (138.4 ± 6.2% vs. 119.1 ± 3.8%, $n = 4$ respectively; $P < 0.05$, Student's t test; Fig. 1A–C). IL-1β (1 ng/mL) application did not alter the baseline EPSP amplitude through the experiment. Next, we examined whether IL-1β impaired NMDA-independent LTP at mossy fiber-CA3 synapses in the same manner. Mossy fiber LTP is likely to be attenuated throughout the time course; therefore, we compared the $EPSP_1$ amplitude 30 min after HFS with that of the baseline as reported previously (3). Unlike SC-CA1 pathways, IL-1β (1 ng/mL) applied for 30 min did not affect mossy fiber LTP (155.0 ± 15.6% vs. 161.2 ± 19.8% of EPSP amplitude 30 min after HFS compared to baseline, $n = 8$ respectively; $P > 0.05$, Mann-Whitney U test; Fig. 2A–C). Finally, we examined the effects of IL-1β on NMDA receptor-dependent LTP at A/C fiber-CA3 synapses. Although baseline EPSPs were slightly elevated after the application of BSA or IL-1β in both the control and IL-1β groups, the difference was not statistically significant. A/C fiber LTP 60 min after HFS was significantly impaired by the application of IL-1β (1 ng/mL) for 30 min (160.9 ± 7.4% vs. 134.3 ± 9.1% of EPSP amplitude 60 min after HFS compared to baseline, $n = 6$ respectively; $P < 0.05$, Student's t test; Fig. 3A–C).

DISCUSSION

To our knowledge, the present results provide the first evidence of synapse-specific effects of IL-1β on LTP in acute mouse hippocampal slices. IL-1β (1 ng/mL) application for 30 min only impaired NMDA receptor-dependent LTP at SC-CA1 and A/C fiber-CA3 synapses. Although we used mice of the same

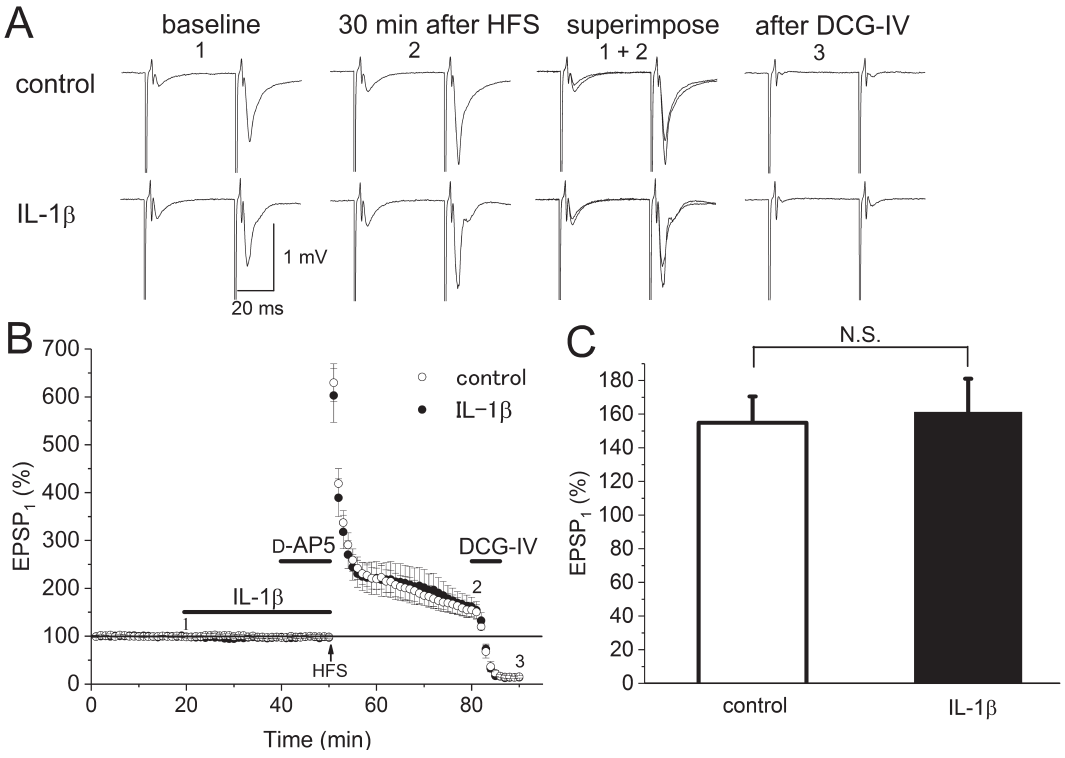

Fig. 2 IL-1β does not affect mossy fiber LTP. (**A**) Effect of IL-1β application (1 ng/mL) on LTP at mossy fiber-CA3 synapses and representative original traces 10 min after DCG-IV application (1 μM). (**B**) Time course of EPSP amplitudes in the above experiment. LTP was induced with a train of 100 Hz for 1 s. Representative examples in A were sampled at the time points labeled by the numbers. (**C**) Percentage of mean EPSP amplitude 30 min after LTP induction against baseline in the control and IL-1β groups. Data are expressed as mean ± SEM. HFS: high-frequency stimulation.

age and a two-fold higher IL-1β concentration than that used previously (11), the effect on mossy fiber LTP was opposite to what Katsuki *et al.* described. Plasticity at mossy fibers that synapse onto CA3 pyramidal cells is fundamentally different from plasticity at other excitatory hippocampal synapses (19), which could explain the different effects of IL-1β on LTP at mossy fiber synapses. Because Katsuki *et al.* did not use DCG-IV or assess any other features of short-term plasticity at mossy fiber-CA3 synapses to confirm the pure mossy fiber signals, it is possible that NMDA receptor-dependent potentiation at the CA3 recurrent collaterals was contaminated in their study.

While we used 1 ng/mL IL-1β in the current study, it is difficult to determine precise synaptic concentrations under pathological conditions (27). Most investigators consider concentration of IL-1β in picograms per milliliters to be healthy (1, 23, 25), and this is essential for sustained LTP expression (25). Meanwhile, IL-1β in CSF from Alzheimer disease patients, who exhibit immune dysfunction in the brain, is about 130 pg/mL (4). Therefore, we considered that 1 ng/mL IL-1β was sufficient to act pathologically even though the synaptic concentrations of neurotransmitters and neuromodulators might be much higher than extracellular levels (29).

The mechanisms by which IL-1β impairs hippocampal LTP remain to be fully elucidated (14). The biological effects of IL-1β are thought to be mediated exclusively through type I IL-1 receptors (20) that are present at high levels in the hippocampus (8, 21). Several studies have shown that members of the mitogen-activated protein kinase (MAPK) family such as p38 and c-Jun N-terminal kinase (c-JNK) are activated by IL-1β via myeloid differentiation factor (MyD) 88 (20), ultimately inhibiting LTP in dentate gyrus (6, 9, 12, 30). Similarly, Coogan *et al.* reported that 1 ng/mL IL-1β depressed NMDA-EPSPs in the dentate gyrus (6) and that this inhibitory effect was attenuated by application of the p38 MAPK inhibitor SB203580. These findings are in accordance with our results demonstrating that IL-1β inhibited only NMDA receptor-dependent LTP. The simplest possible explanation for this phenomenon is that p38 MAPK directly depresses the function of

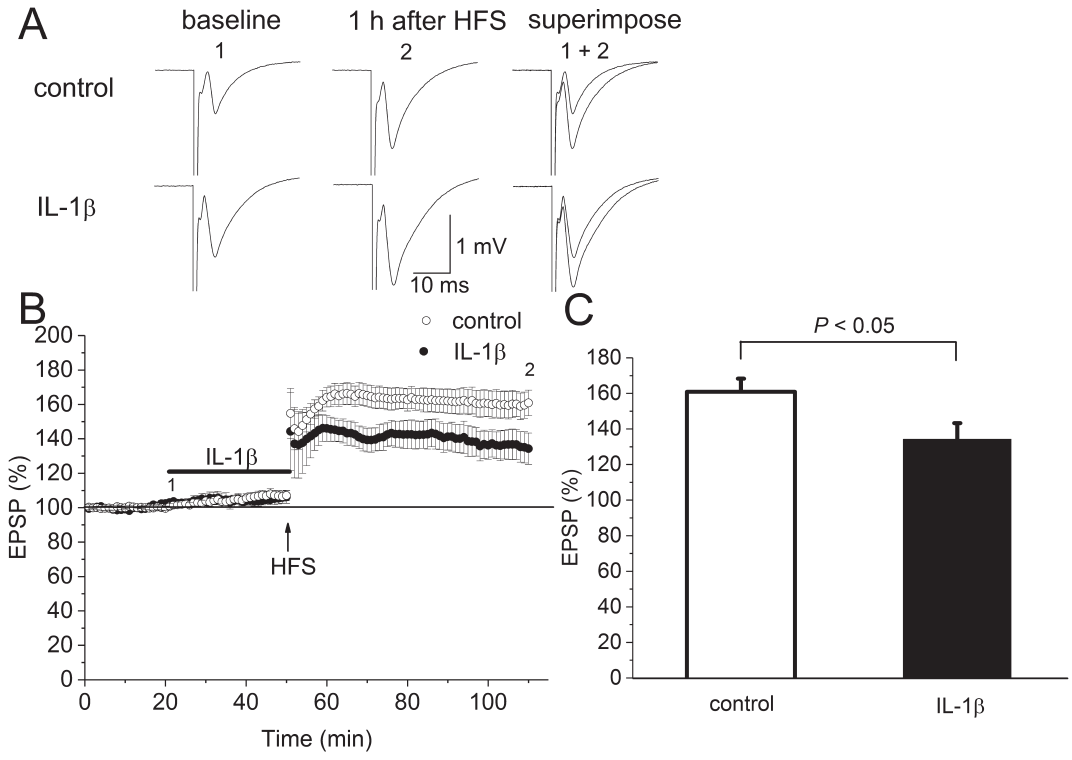

Fig. 3 IL-1β impairs A/C fiber-CA3 LTP. (**A**) Effect of IL-1β application (1 ng/mL) on LTP at A/C fiber-CA3 synapses. (**B**) Time course of EPSP amplitude in the above experiment. LTP was induced with the same protocol shown in Fig. 1. Representative examples in A were sampled at the time points labeled by the numbers. (**C**) Percentage of mean EPSP amplitude 60 min after LTP induction against baseline in the control and IL-1β groups. Data are expressed as mean ± SEM. HFS: high-frequency stimulation.

NMDA receptors and impairs LTP. However, some reports using primary cultured hippocampal neurons indicated that IL-1β increased NMDA-mediated Ca^{2+} current (31, 32); therefore, the effects of IL-1β on NMDA receptors need to be further studied. In addition to the effects on NMDA receptors, IL-1β also affects brain-derived neurotrophic factor-induced signaling in a p38-dependent manner (29), suggesting that various IL-1β-induced signaling events affect LTP induction.

It is important to consider some limitations when interpreting our results. First, we used immature mice (4–6 weeks old) as employed in a previous report (11). One study showed that neuroinflammatory responses and hippocampal LTP deficits could be age-dependent (13); therefore, different results might be obtained in slices from aged mice. Secondly, we only used one IL-1β concentration in our experiments. Although we assumed that 1 ng/mL IL-1β was sufficient to act pathologically as described above, higher concentrations of IL-1β could exert different results because the effect of IL-1β on LTP is reportedly dose dependent (14).

In conclusion, our findings provide the first evidence of a synapse-specific effect of IL-1β on NMDA receptor-dependent LTP in the mouse hippocampus. This clue could be useful for elucidating the mechanisms by which IL-1β modulates synaptic plasticity.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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