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Lipopolysaccharide inhibits induction of long-term potentiation and depression in the rat hippocampal CA1 area

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Abstract

We examined the effects of lipopolysaccharide, a bacterial endotoxin, on synaptic plasticity in the rat hippocampal CA1 area in vitro. Lipopolysaccharide suppressed the induction of long-term potentiation elicited by tetanic stimulation and long-term depression, elicited by low-frequency stimulation of Schaffer collateral–commissural fibres at 10 and 50 μg/ml, respectively. Lipid A (1 μg/ml), the biologically active component of lipopolysaccharide, mimicked the effects of 10 μg/ml lipopolysaccharide on long-term potentiation and depression. Nifedipine, an L-type voltage-sensitive Ca^{2+} channel antagonist, did not influence the induction of long-term potentiation and depression, whereas a high concentration of extracellular calcium enabled long-term potentiation induction in the presence of 10 μg/ml lipopolysaccharide.

The NMDA receptor antagonist D,L-2-amino-5-phosphonovaleric acid (APV, 50 μM), nifedipine (10 μM) or lipopolysaccharide (10 or 50 μg/ml) partially reduced the magnitude of tetraethylammonium-induced long-term potentiation. Nifedipine combined with lipopolysaccharide completely blocked tetraethylammonium-induced long-term potentiation. Whole-cell voltage clamp recordings showed that lipopolysaccharide suppressed NMDA receptor-mediated excitatory postsynaptic currents (EPSCs). Our results indicate that lipopolysaccharide acutely modifies synaptic plasticity by blocking Ca^{2+} entry through NMDA receptors, suggesting a possible mechanism for the amnesic action of bacterial infection. © 2001 Published by Elsevier Science B.V.

Keywords: Lipopolysaccharide; Long-term potentiation; Long-term depression; NMDA receptor; Hippocampus

1. Introduction

The central nervous system possesses its own immune/inflammatory response system of microglia and astrocytes, which act like macrophages in bacterial infections (Perry et al., 1993) that can cause behavioural disturbances, including impairment of learning and memory ability (Pugh et al., 1998; Ma and Zhu, 1997). Lipopolysaccharide, an endotoxin from the cell walls of gram-negative bacteria, is a major immune/inflammatory agent that is widely used in the central nervous system as well as in the periphery. One of the host defence strategies during lipopolysaccharide challenge is the induction of various cytokines, such as interleukin-1β, interleukin-6, interferon-α, and tumour necrosis factor-α. In the central nervous system, intraperitoneal injection of lipopolysaccharide results in increased concentrations of interleukin-1β messenger RNA in the hippocampus, pituitary, and hypothalamus in mice. The kinetics of messenger RNA expression of these cytokines are different than those observed in the periphery, e.g., spleen, suggesting that there is local synthesis of proinflammatory cytokines in certain brain structures (Gabellec et al., 1995).

It has also been reported that lipopolysaccharide itself exerts various influences on the central nervous system through activation of various cellular and biochemical events. For example, direct injection of lipopolysaccharide into the rat cerebral cortex and hippocampus induces nitric oxide synthase, which in turn produces nitric oxide (Yamada et al., 1999). Furthermore, nitric oxide synthase messenger RNA, protein, and nitric oxide products are also induced in cultured microglia by lipopolysaccharide (Fiebich et al., 1998).

While the immunological and biochemical properties of lipopolysaccharide in vivo and in cultured cells have been studied in great detail, little is known about its action on

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synaptic function. In one study, acute local perfusion of lipopolysaccharide resulted in a modification of the firing rate of thermosensitive neurons in hypothalamic slices (Nakashima et al., 1985). In another study, lipopolysaccharide induced a very rapid and sustained release of glutamate in rat cortical slices (Wang and White, 1999). Glutamate plays an important role in the central nervous system as a major excitatory neurotransmitter, and causes excitotoxicity when released extensively. Its AMPA and NMDA receptors are critically involved in synaptic transmission and plasticity. Therefore, it is possible that changes in synaptic transmission may follow a bacterial infection, because released glutamate can act at AMPA receptors, which in turn causes activation of NMDA receptors.

Based on the above earlier work, we investigated in the present study the effects of lipopolysaccharide on synaptic transmission and plasticity, for example long-term potentiation and depression, in the CA1 area of rat hippocampal slices. Long-term potentiation and depression have been viewed as two major modifications of synaptic efficacy underlying learning and memory (for review see Bear, 1999; Malenka and Nicoll, 1999). Our results demonstrated that lipopolysaccharide, a bacterial endotoxin, suppresses long-term potentiation and depression through blockade of NMDA receptor-mediated synaptic activity.

2. Materials and methods

2.1. Recording of field excitatory postsynaptic potentials

Sprague–Dawley rats (4–6-weeks old) were decapitated under halothane anaesthesia, and their brains were rapidly removed and placed in ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM) 126 NaCl, 3 KCl, 1.4 KH_2PO_4, 1.8 $CaCl_2 \cdot 2H_2O$, 1.3 $MgSO_4$, 23 $NaHCO_3$, and 10 D-glucose, continuously bubbled with 95% O_2/5% CO_2. Transverse hippocampal slices (400-μm thick) were prepared with a McIlwain-type chopper (Hugo Sachs Elektronik, March, Germany) and incubated for at least 1 h in a chamber containing oxygenated aCSF before recording. Slices were then transferred to a submerged recording chamber at 30 °C, which was perfused with aCSF saturated with 95% O_2/5% CO_2 at a flow rate of 1–2 ml/min. Field excitatory postsynaptic potentials (EPSPs) were evoked by stimulation of Schaffer collateral–commissural fibres with monophasic square-wave pulses (duration, 100 μs), delivered through a bipolar electrode and were recorded with a glass electrode (resistance, 2–5 MΩ) filled with aCSF using a DAM80 differential amplifier (World Precision Instruments, Sarasota, FL). For baseline recording, field EPSPs were evoked every 30 s, and stimu-

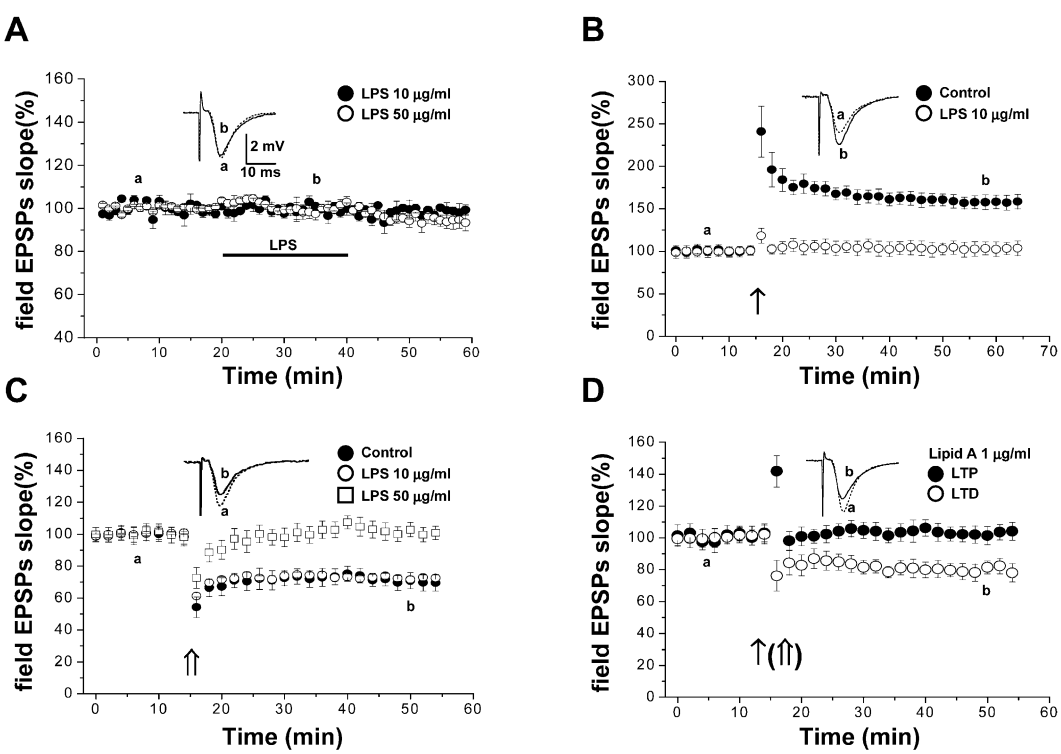

Fig. 1. Effects of lipopolysaccharide and lipid A on field potential long-term potentiation and depression. (A) Effects of lipopolysaccharide on synaptic transmission. Bath application of 10 and 50 μg/ml lipopolysaccharide for 20 min induced no change in field EPSPs. (B) Long-term potentiation induction in the absence or presence of 10 μg/ml lipopolysaccharide. The initial slopes of field EPSPs were normalized to the baseline value before tetanic stimulation. Test stimuli were applied every 30 s. (C) Long-term depression induction in the absence or presence of lipopolysaccharide (10 or 50 μg/ml). (D) Long-term potentiation and depression induction in the presence of 1 μg/ml lipid A. Thick horizontal black bar: duration of lipopolysaccharide application; single arrow: time of tetanic stimulation; double arrow: low-frequency stimulation. Representative traces of field EPSPs at the time point indicated by the corresponding letters are shown above the graphs.

lus intensity was adjusted to yield responses of similar size in different slices. Field potential long-term potentiation was induced with three trains of 100-Hz stimulation lasting 1 s at intervals of 20 s. Long-term depression was evoked by 900 stimuli at a frequency of 1 Hz for 15 min. Long-term potentiation and depression were quantified by averaging the field EPSPs slopes from five consecutive responses, 40 min following tetanic stimulation and low-frequency stimulation, respectively. Results are expressed as percentages relative to control baseline. Responses were digitised on-line and analysed using the NAC software (Electek, Irvine, CA). All data are expressed as means ± S.E.M. Statistical analysis was performed using Student's *t*-test with significance set at $P < 0.05$.

2.2. Whole-cell voltage clamp recordings

Hippocampal slices with a thickness of 200 μm were prepared from 3- to 4-week-old rats. Slices were transferred to a Delta T dish (Bioptechs, Butler, PA) at 30 °C, and continuously perfused at a rate of 2–3 ml/min with Mg^{2+}-free aCSF oxygenated with 95% O_2/5% CO_2. In a standard bath solution, magnesium was removed for amplification of NMDA receptor-mediated currents. For isolation of NMDA receptor-mediated currents, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), bicuculline methiodide (10 μM), and saclofen (100 μM) were always present to block AMPA/kainate receptor- and γ-aminobutyric acid (GABA) receptor-mediated currents. Whole-cell recordings were made from visually identified CA1 pyramidal cells using a ×40 water immersion objective attached to an upright microscope (model BX-50WI, Olympus, Tokyo). The recording electrodes were fabricated from thick-walled borosilicate glass and had a resistance of 5–10 MΩ. The composition of the pipette solution for recording was (in mM) 170 K^+-gluconate, 10 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid, 10 NaCl, 2 $MgCl_2$, 10 ethylene glycol bis(β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid, and 3.5 Mg^{2+}-ATP (pH 7.2 adjusted with NaOH). Ionic currents were recorded with an Axopatch 200A patch-clamp amplifier (Axon Instruments, Foster City, CA). Stimulation and on-line acquisition were performed using a signal generator (WPI) and pCLAMP program (Axon Instruments). Signals were filtered at 2 kHz and digitised at 20 kHz. For stimulation of Schaffer collateral–commissural fibres in the hippocampus, a bipolar tungsten electrode was used.

2.3. Drugs

Saclofen, bicuculline methiodide, D,L-2-amino-5-phosphonovaleric acid (APV) and CNQX were purchased from Research Biochemicals International (Natick, MA). All other chemicals including lipopolysaccharide and lipid A were from Sigma (St. Louis, MO).

3. Results

3.1. Lipopolysaccharide suppresses synaptic plasticity

The initial slope of field EPSPs was not significantly modified during a 20-min treatment with 10 and 50 μg/ml lipopolysaccharide compared to baseline (10 μg/ml, 102.1 ± 1.4%; 50 μg/ml, 101.6 ± 2.3%, $n = 5$, Fig. 1A). In order to study the effects of lipopolysaccharide on synaptic plasticity, long-term potentiation and depression were examined in the CA1 area of rat hippocampal slices. Stable baseline recordings were obtained for 20 min before treatment with lipopolysaccharide, which was perfused for 15 min before tetanus or low-frequency stimulation. Tetanic stimulation of the Schaffer collateral–commissural fibres produced long-term potentiation of field EPSPs in the CA1 area in 11 slices from 9 rats. The normalised field EPSPs

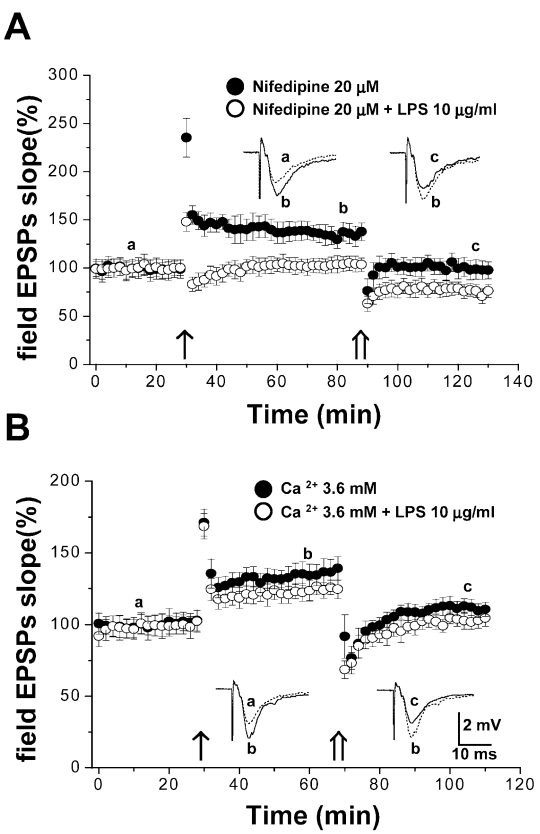

Fig. 2. Effects of lipopolysaccharide on long-term potentiation and depression in the presence of 20 μM nifedipine, an L-type Ca^{2+} channel blocker, or a high concentration of Ca^{2+} (3.6 mM) in artificial cerebrospinal fluid (aCSF). (A) Long-term potentiation induction by tetanic stimulation and subsequent depotentiation following low-frequency stimulation in the presence of 20 μM nifedipine. Addition of 10 μg/ml lipopolysaccharide inhibited long-term potentiation after tetanic stimulation, but low-frequency stimulation resulted in long-term depression to an extent similar to that of the control. (B) Long-term potentiation induction and depotentiation in the presence of a high concentration of Ca^{2+} in aCSF. High Ca^{2+} aCSF blocked the lipopolysaccharide inhibitory effect on long-term potentiation induction.

slope was 158.1 ± 3.3% of baseline value at 50 min after tetanus. In the presence of 10 μg/ml lipopolysaccharide, the degree of long-term potentiation was decreased to 104 ± 1.4% ($n = 7$, $P < 0.01$, Fig. 1B). We further tested the effects of lipopolysaccharide on long-term depression, which reflects a sustained decrease in synaptic efficiency. Low-frequency stimulation (1 Hz, 15 min) applied to the Schaffer collateral–commissural fibres induced a long-lasting reduction in CA1 field EPSPs in eight slices from six rats. The magnitude of the long-term depression in 10 μg/ml lipopolysaccharide-treated slices was 23.4 ± 2.1% ($n = 7$), similar to that in the control slices (28.6 ± 3.4%, $n = 7$, Fig. 1C). This selective inhibition by 10 μg/ml lipopolysaccharide of long-term potentiation could be due to a specific effect on cellular pathways pertaining to potentiation. However, since long-term depression can be triggered under conditions such as low extracellular Ca^{2+} concentration and insufficient activation of NMDA receptors, which is below the threshold for inducing synaptic potentiation (Dudek and Bear, 1992; Mulkey and Malenka, 1992), it is also possible that the induction of long-term depression is inhibited following complete blockade of NMDA receptors and Ca^{2+} influx. The latter possibility was tested using a high dose of lipopolysaccharide, in anticipation of blocking long-term depression. At a dose of 50 μg/ml lipopolysaccharide, the amplitude of baseline field EPSPs in response to a moderate stimulus intensity did not change. Interestingly, the induction of long-term depression was blocked (102.4 ± 2.1%, $n = 5$, Fig. 1C). We also tested the effects of the biologically active component of lipopolysaccharide, lipid A, to determine whether it could mimic the effect of lipopolysaccharide. Lipid A inhibited the expression of long-term potentiation (104.3 ± 1.4%, $n = 6$), but partially attenuated long-term depression (19.0 ± 2.4%, $n = 6$) at a dose of 1 μg/ml, suggesting that the effects of lipopolysaccharide on long-term potentiation and depression are not non-specific actions irrelevant to the action of lipopolysaccharide endotoxin (Fig. 1D).

3.2. Inhibitory effects of lipopolysaccharide are modified by Ca^{2+} ions

The results of the above experiments (Fig. 1) suggest that lipopolysaccharide suppresses the induction of long-term potentiation and depression, possibly through a com-

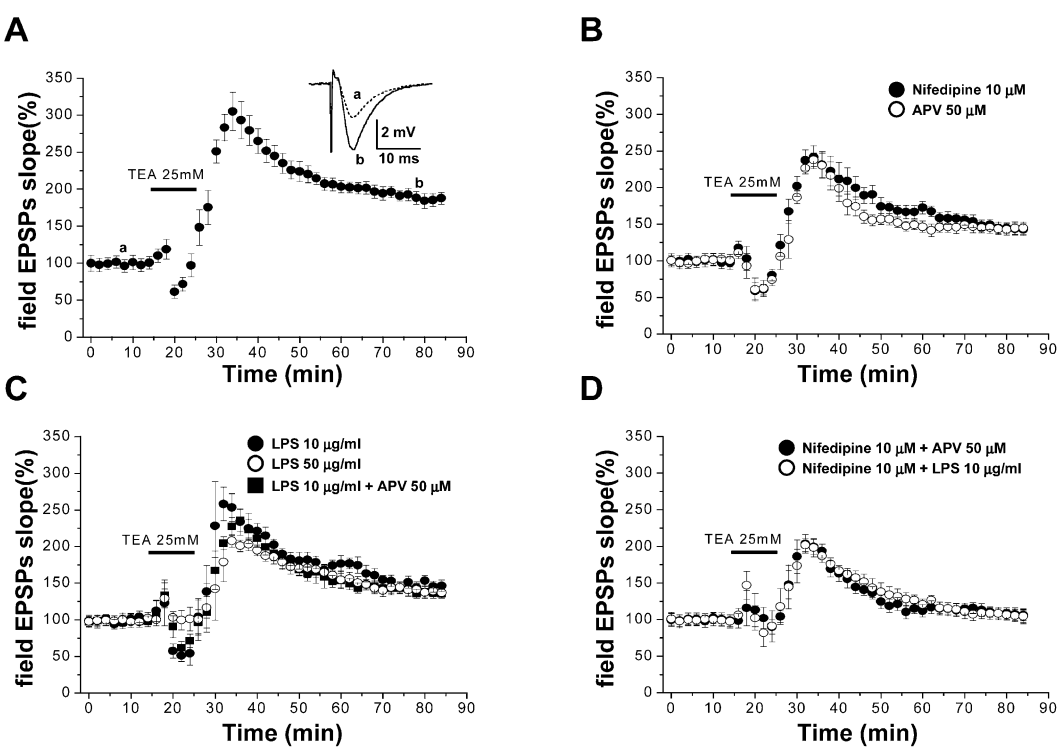

Fig. 3. Effects of lipopolysaccharide on tetraethylammonium-induced long-term potentiation under various conditions. (A) Induction of long-term potentiation following application of 25 mM tetraethylammonium for 10 min. The mean value of potentiation at 60 min after washout of tetraethylammonium was 188 ± 3.2%. The superimposed traces above the graph illustrate field EPSPs at the time period indicated by letters. (B) Partial reduction of tetraethylammonium-induced long-term potentiation by 10 μM nifedipine or 50 μM APV. (C) Partial blockade of tetraethylammonium-induced long-term potentiation by lipopolysaccharide (10 or 50 μg/ml). Note the lack of additive effect of APV and lipopolysaccharide. APV (50 μM) and lipopolysaccharide (10 μg/ml) were simultaneously added 15 min before tetraethylammonium treatment. (D) Complete inhibition of tetraethylammonium-induced long-term potentiation by simultaneous addition of 50 μM APV or 10 μg/ml lipopolysaccharide and 10 μM nifedipine 15 min before tetraethylammonium application.

mon mechanism. We hypothesized that this mechanism involves the influx of Ca^{2+} ions, because Ca^{2+} ions are required as a primary initiation signal for the induction of long-term depression as well as long-term potentiation (Lynch et al., 1983; Mulkey and Malenka, 1992). In the next series of experiments, we tested the effects of lipopolysaccharide under different conditions of Ca^{2+} influx. Furthermore, to ascertain that the effects of lipopolysaccharide on long-term potentiation and depression were not due to variability among slices, we first induced long-term potentiation and then delivered a low-frequency stimulation in the same slice after 40–60 min of tetanic stimulation. In the presence of either an L-type Ca^{2+} channel blocker, nifedipine (20 μM), or a high concentration of extracellular Ca^{2+} (3.6 mM) compared to 1.8 mM of normal aCSF, both long-term potentiation and depression were induced in control slices ($134.2 \pm 1.2\%$ and $29.6 \pm 4.4\%$, $n = 5$, for nifedipine; $132.3 \pm 3.4\%$ and $25.7 \pm 2.5\%$, $n = 6$, for high Ca^{2+}) (Fig. 2). However, long-term potentiation induction in the presence of nifedipine failed to occur in lipopolysaccharide-treated slices ($103.2 \pm 1.1\%$, $n = 5$, Fig. 2A). In contrast, the extent of long-term depression under both conditions ($22.4 \pm 1.3\%$ and $21.3 \pm 2.8\%$, $n = 5$, for nifedipine and high Ca^{2+}, respectively) and the extent of long-term potentiation in the presence of a high extracellular concentration of Ca^{2+} ($124.2 \pm 1.5\%$, $n = 5$) were not significantly reduced by lipopolysaccharide (Fig. 2B). Since blockade of voltage-gated Ca^{2+} channels had no effect on the action of lipopolysaccharide on long-term potentiation induction, and a high concentration of extracellular Ca^{2+} inhibited the effect of lipopolysaccharide on long-term potentiation, it was postulated that lipopolysaccharide blocked long-term potentiation induction by acting on NMDA receptors, which are one of the major pathways for calcium entry. If this were true, lipopolysaccharide would not block NMDA receptor-independent long-term potentiation.

3.3. Lipopolysaccharide inhibits NMDA receptor-dependent long-term potentiation

Activation of voltage-gated Ca^{2+} channels and NMDA receptors is involved in tetraethylammonium-induced long-term potentiation as a mechanism of Ca^{2+} influx into postsynaptic cells (Huber et al., 1995). Treatment of slices with 25 mM tetraethylammonium, a K^+ channel blocker, for 10 min caused a transient depression of the field EPSPs slope, followed by a potentiation after washout of tetraethylammonium. The degree of tetraethylammonium-induced long-term potentiation was $188.1 \pm 3.2\%$ ($n = 8$) at 60 min after washout compared to the baseline (Fig. 3A), and this potentiation was partially blocked by 10 μM nifedipine ($146.7 \pm 1.6\%$, $n = 6$) and by 50 μM APV ($144.2 \pm 3.3\%$, $n = 6$), an NMDA receptor antagonist (Fig. 3B). Complete suppression of long-term potentiation induction was noted only during application of nifedipine and APV ($107.7 \pm 0.9\%$, $n = 5$, $P > 0.05$, Fig. 3D). These results are consistent with those of Huber et al. (1995), confirming that tetraethylammonium-induced long-term potentiation is mediated through Ca^{2+} influx via voltage-

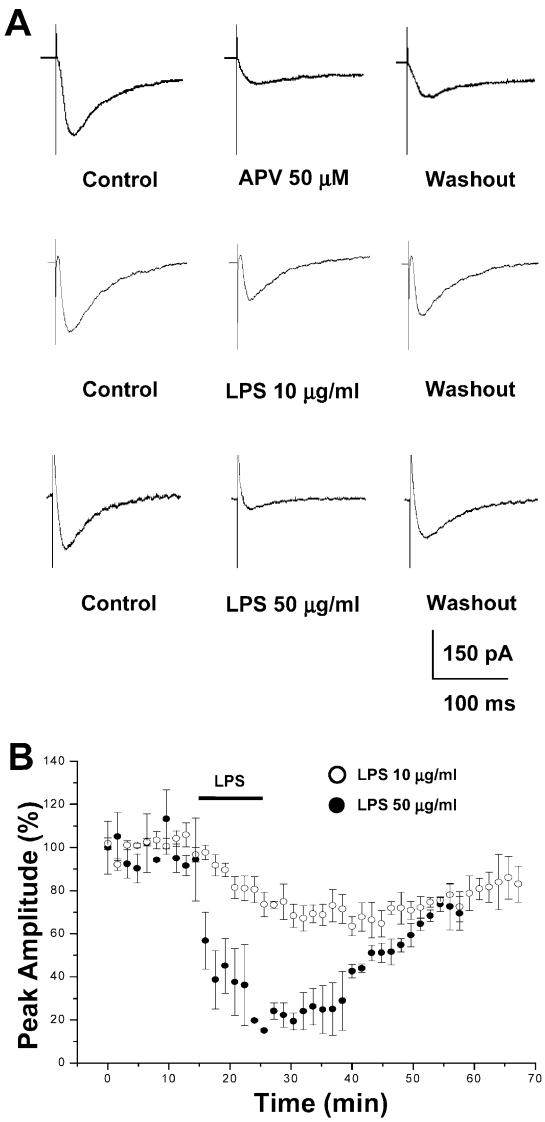

Fig. 4. Action of lipopolysaccharide on D,L-2-amino-5-phosphonovaleric acid (APV)-sensitive evoked excitatory postsynaptic currents (EPSCs) recorded in CA1 pyramidal cells. APV-sensitive EPSCs were elicited in the presence of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), bicuculline methiodide (10 μM), and saclofen (100 μM) in Mg^{2+}-free aCSF. (A) EPSCs traces before and after application of 50 μM APV and lipopolysaccharide (10 or 50 μg/ml). The stimulus artefact is partially truncated. (B) Pooled normalized EPSCs plotted against time, showing the effect of bath application of lipopolysaccharide (10 μg/ml, $n = 5$, open circles; 50 μg/ml, $n = 4$, closed circles). Each point represents the mean ± S.E.M. of five successive EPSCs, normalized to the mean of all responses recorded in the control period. Holding potential was at −40 mV.

gated Ca^{2+} channels and NMDA receptors, and that activation of voltage-gated Ca^{2+} channels is sufficient for long-term potentiation induction under conditions of NMDA receptor blockade. As lipopolysaccharide blocked the tetanus-induced long-term potentiation, 10 μg/ml lipopolysaccharide also inhibited tetraethylammonium-induced long-term potentiation, being similar to APV; the magnitude of long-term potentiation was reduced to 146.5 ± 3.4% ($n = 5$, Fig. 3C), and after the addition of nifedipine, lipopolysaccharide completely suppressed tetraethylammonium-induced long-term potentiation (106.8 ± 0.6%, $n = 7$, Fig. 3D). Addition of APV in the presence of lipopolysaccharide failed to further suppress tetraethylammonium-induced long-term potentiation (137.2 ± 4.7%, $n = 5$, Fig. 3C), confirming that lipopolysaccharide exerts its effect through an NMDA receptor-dependent mechanism. Furthermore, even a high dose of lipopolysaccharide (50 μg/ml) could not completely block tetraethylammonium-induced long-term potentiation (140.4 ± 7.9%, $n = 8$, Fig. 3C).

3.4. Lipopolysaccharide reduces NMDA receptor-mediated excitatory postsynaptic currents (EPSCs)

To test the effects of lipopolysaccharide on NMDA receptors, pharmacologically isolated NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) were directly measured, using whole-cell voltage clamp recordings, in CA1 pyramidal cells of hippocampal slices perfused with Mg^{2+}-free aCSF in the presence of CNQX (10 μM), bicuculline methiodide (10 μM), and saclofen (100 μM), which blocked AMPA, $GABA_A$, and $GABA_B$ receptors, respectively (Fig. 4). APV-sensitive EPSCs were elicited with 20–50 ms of peak latency in response to single electrical stimulation of Schaffer collateral–commissural fibres. This response was blocked by 50 μM APV. Lipopolysaccharide reduced APV-sensitive EPSCs in a dose-dependent manner (61.2 ± 4.2% of control amplitude, $n = 5$ in 10 μg/ml and 31.7 ± 14.1%, $n = 4$ in 50 μg/ml).

4. Discussion

The major findings of the present study were that lipopolysaccharide suppressed the induction of hippocampal CA1 long-term potentiation and depression, two different forms of synaptic plasticity, possibly by reducing Ca^{2+} influx through NMDA receptors. Lipopolysaccharide also caused a reduction in NMDA receptor-mediated EPSCs.

Long-term potentiation and depression are two well-established cellular models of synaptic plasticity underlying certain types of learning and memory. Long-term potentiation reflects a long-lasting increase in synaptic efficacy, whereas long-term depression, the functional inverse of long-term potentiation, reflects a decrease in synaptic efficacy. Both CA1 long-term potentiation and depression require NMDA receptor activation for the entry of Ca^{2+} as a primary initiation signal, and different magnitudes and dynamics of Ca^{2+} influx are known to activate different cascades of intracellular enzymatic events, which profoundly influence the forms of the resulting synaptic plasticity (Connor et al., 1999; Coussens and Teyler, 1996). A rapid and large influx of Ca^{2+} into the cytoplasm is crucial for long-term potentiation induction, which eventually activates protein kinases. A slow and modest influx of Ca^{2+} occurs during long-term depression induction. Phosphatase is predominantly implicated in long-term depression.

In the present study, we demonstrated that an increase in extracellular Ca^{2+} from 1.8 to 3.6 mM attenuated the inhibitory effect of lipopolysaccharide on tetanus-induced long-term potentiation. Under the same conditions, tetraethylammonium-induced long-term potentiation was partially suppressed, while tetanus-induced long-term potentiation was completely suppressed by lipopolysaccharide. Since activation of NMDA receptors is required for long-term potentiation induction by tetanic stimulation in the CA1 area and, in contrast, tetraethylammonium-induced long-term potentiation can occur through either activation of NMDA receptors or voltage-gated Ca^{2+} channels, lipopolysaccharide appears to act like APV in controlling NMDA receptor-dependent long-term potentiation. This was supported by the finding that a high dose of lipopolysaccharide (50 μg/ml) failed to completely block tetraethylammonium-induced long-term potentiation. The inhibitory effect of combined treatment with APV and lipopolysaccharide was no more than that of APV or lipopolysaccharide alone. Finally, lipopolysaccharide plus nifedipine suppressed tetraethylammonium-induced long-term potentiation completely, as did APV plus nifedipine.

Since long-term depression as well as long-term potentiation is dependent on the activation of NMDA receptors, and if suppression of long-term potentiation is caused by blockade of NMDA receptors, the question is raised as to why long-term depression induced by low-frequency stimulation was not blocked by 10 μg/ml of lipopolysaccharide. One possible explanation is that 10 μg/ml lipopolysaccharide controls Ca^{2+} influx by blocking NMDA receptors in a differential manner, that is, the rapid and large Ca^{2+} influx is attenuated, while the slow and small Ca^{2+} influx is sustained during partial blockade of NMDA receptors. The level of postsynaptic Ca^{2+} entry during conditioning stimulation is a critical factor in determining the development of either long-term potentiation or depression. In fact, it was shown that brief tetanic stimulation, which normally generates long-term potentiation, could induce long-term depression in the presence of moderate concentrations of APV because of a limited Ca^{2+} entry via NMDA receptors (Cummings et al., 1996).

In support of this explanation, long-term potentiation and depression induced by electrical stimulation were both suppressed by a high dose of lipopolysaccharide, possibly due to a complete blockade of NMDA receptors. However, even a high dose of lipopolysaccharide only partially suppressed tetraethylammonium-induced long-term potentiation, suggesting that lipopolysaccharide does not block voltage-gated Ca^{2+} channels. NMDA receptor-mediated EPSCs in Mg^{2+}-free media were completely suppressed by 50 μg/ml lipopolysaccharide.

To date, most studies of lipopolysaccharide have been carried out with cultured cells or animals, but not with in vitro slice preparations. In cultured cells or in animals, lipopolysaccharide effects were studied over a prolonged time scale, which allows sufficient time for the release of proinflammatory cytokines, changes in gene transcription, and eventual neuronal damage. Under the present conditions with brain slices, lipopolysaccharide effects such as enhancement of glutamate release and inhibition of long-term potentiation induction are much too rapid to involve gene transcription and protein synthesis. For suppression of long-term potentiation and depression, a 15-min pretreatment was sufficient. Therefore, the effects of lipopolysaccharide on long-term potentiation are unlikely to involve gene transcriptional process. Nor is neuronal damage caused by lipopolysaccharide a possible factor, because neuronal damage is often reported in the late phase of infection. However, the present study does not rule out the possible involvement of cytokine release in response to the lipopolysaccharide insult. Accumulating results show that cytokines are involved in synaptic plasticity in the mammalian brain. Perfusion with interleukin-1β, interleukin-2, or interleukin-6 suppresses the induction of long-term potentiation in the hippocampus (Li et al., 1997; Bellinger et al., 1993; Tancredi et al., 1990). Interleukin-1β suppresses tetanus- or tetraethylammonium-induced long-term potentiation in CA1, CA3, and dentate gyrus areas (Coogan and O'Connor, 1999; Cunningham et al., 1996; Katsuki et al., 1990). Furthermore, reduced long-term potentiation was reported in hippocampal slices from transgenic mice that overexpressed interleukin-6 (Bellinger et al., 1995). Nevertheless, to our knowledge, there are no studies that have previously reported the release of cytokines following acute treatment of in vitro brain preparations with any type of endotoxin. Whether the effects of lipopolysaccharide on synaptic transmission and plasticity can be mediated through cytokines associated with immune/inflammatory responses remains to be determined.

Acknowledgements

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References

Bear, M.F., 1999. Homosynaptic long-term depression: a mechanism for memory? Proc. Natl. Acad. Sci. U. S. A. 96, 9457–9458.

Bellinger, F.P., Madamba, S.G., Siggins, G.R., 1993. Interleukin 1β inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. Brain Res. 628, 227–234.

Bellinger, F.P., Madamba, S.G., Campbell, I.L., Siggins, G.R., 1995. Reduced long-term potentiation in the dentate gyrus of transgenic mice with cerebral overexpression of interleukin-6. Neurosci. Lett. 198, 95–98.

Connor, J.A., Petrozzino, J., Pozzo-Miller, L.D., Otani, S., 1999. Calcium signals in long-term potentiation and long-term depression. Can. J. Physiol. Pharmacol. 77, 722–734.

Coogan, A.N., O'Connor, J.J., 1999. Interleukin-1β inhibits a tetraethylammonium-induced synaptic potentiation in the rat dentate gyrus in vitro. Eur. J. Pharmacol. 374, 197–206.

Coussens, C.M., Teyler, T.J., 1996. Protein kinase and phosphatase activity regulate the form of synaptic plasticity expressed. Synapse 24, 97–103.

Cummings, J.A., Mulkey, R.M., Nicoll, R.A., Malenka, R.C., 1996. Ca^{2+} signalling requirements for long-term depression in the hippocampus. Neuron 16, 825–833.

Cunningham, A.J., Murray, C.A., O'Neill, L.A., Lynch, M.A., O'Connor, J.J., 1996. Interleukin-1β (IL-1β) and tumour necrosis factor (TNF) inhibit long-term potentiation in the rat dentate gyrus in vitro. Neurosci. Lett. 203, 17–20.

Dudek, S.M., Bear, M.F., 1992. Homosynaptic long-term depression in the area CA1 hippocampus and effects of *N*-methyl-D-aspartate receptor blockade. Proc. Natl. Acad. Sci. U. S. A. 89, 4363–4367.

Fiebich, B.L., Butcher, R.D., Gebicke-Haerter, P.J., 1998. Protein kinase C-mediated regulation of inducible nitric oxide synthase expression in cultured microglial cells. J. Neuroimmunol. 92, 170–178.

Gabellec, M.M., Griffais, R., Fillion, G., Haour, F., 1995. Expression of interleukin-1α, interleukin-1β and interleukin-1 receptor antagonist messenger RNA in mouse-brain-regulation by bacterial lipopolysaccharide (LPS) treatment. Mol. Brain Res. 31, 122–130.

Huber, K.M., Mauk, M.D., Kelly, P.T., 1995. Distinct LTP induction mechanisms: contribution of NMDA receptors and voltage-dependent calcium channels. J. Neurophysiol. 73, 270–279.

Katsuki, H., Nakai, S., Hirai, Y., Akaji, K., Satoh, M., 1990. Interleukin-1β inhibits long-term potentiation in the CA3 region of mouse hippocampal slices. Eur. J. Pharmacol. 181, 323–328.

Li, A.J., Katafuchi, T., Oda, S., Hori, T., Oomura, Y., 1997. Interleukin-6 inhibits long-term potentiation in rat hippocampal slices. Brain Res. 748, 30–38.

Lynch, G., Larson, J., Kelso, S., Barrionuevo, G., Schottler, F., 1983. Intracellular injections of EGTA block induction of hippocampal long-term potentiation. Nature 305, 719–721.

Ma, T.C., Zhu, X.Z., 1997. Suppression of lipopolysaccharide-induced impairment of active avoidance and interleukin-6-induced increase of prostaglandin E2 release in rats by indomethacin. Arzneim.-Forsch. 47, 595–597.

Malenka, R.C., Nicoll, R.A., 1999. Long-term potentiation—a decade of progress? Science 285, 1870–1874.

Mulkey, R.M., Malenka, R.C., 1992. Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9, 967–975.

Nakashima, T., Hori, T., Kiyohara, T., Shibata, M., 1985. Effects of endotoxin and sodium salicylate on the preoptic thermosensitive neurons in tissue slices. Brain Res. Bull. 15, 459–463.

Perry, V.H., Anderson, P.B., Gordon, S., 1993. Macrophages and inflammation in the central nervous system. Trends Neurosci. 16, 268–273.

Pugh, C.R., Kumagawa, K., Fleshner, M., Watkins, L.R., Maier, S.F., Rudy, J.W., 1998. Selective effects of peripheral lipopolysaccharide administration on contexual and auditory-cue fear conditioning. Brain, Behav., Immun. 12, 212–229.

Tancredi, V., Zona, C., Velotti, F., Eusebi, F., Santoni, A., 1990. Interleukin-2 suppresses established long-term potentiation and inhibits its induction in the rat hippocampus. Brain Res. 525, 149–151.

Wang, Y.S., White, T.D., 1999. The bacterial endotoxin lipopolysaccharide causes rapid inappropriate excitation in rat cortex. J. Neurochem. 72, 652–660.

Yamada, K., Komori, Y., Tanaka, T., Senzaki, K., Nikai, T., Sugihara, H., Kameyama, T., Nabeshima, T., 1999. Brain dysfunction associated with an induction of nitric oxide synthase following an intracerebral injection of lipopolysaccharide in rats. Neuroscience 88, 281–294.