THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 278, No. 21, Issue of May 23, pp. 19453–19462, 2003
Printed in U.S.A.

Activation of p38 Plays a Pivotal Role in the Inhibitory Effect of Lipopolysaccharide and Interleukin-1β on Long Term Potentiation in Rat Dentate Gyrus*

Received for publication, February 24, 2003
Published, JBC Papers in Press, February 27, 2003, DOI 10.1074/jbc.M301938200

Áine Kelly‡, Emily Vereker, Yvonne Nolan, Marcella Brady, Claire Barry, Christine E. Loscher§, Kingston H. G. Mills§, and Marina A. Lynch

From the Trinity College Institute of Neuroscience, Department of Physiology, and §Department of Biochemistry, Trinity College, Dublin 2, Ireland

Lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria, has been shown to induce profound changes both peripherally and centrally. It has recently been reported that intraperitoneal injection of LPS inhibited long term potentiation (LTP) in perforant path-granule cell synapses and that this effect was coupled with an increase in the concentration of the proinflammatory cytokine, interleukin-1β (IL-1β). The LPS-induced effects were abrogated by inhibition of caspase-1, suggesting that IL-1β may mediate the effects of LPS. Here we report that the inhibition of LTP induced by LPS and IL-1β was coupled with stimulation of the stress-activated protein kinase p38 in hippocampus and entorhinal cortex and that this effect was abrogated by the p38 inhibitor SB203580, while the effect of LPS was markedly attenuated in C57BL/6 IL-1RI$^{-/-}$ mice. The data also indicate that activation of the transcription factor, nuclear factor κB (NFκB), may play a role, since the inhibitory effect of LPS and IL-1β on LTP was attenuated by the NFκB inhibitor, SN50; consistently, LPS and IL-1β led to activation of NFκB in entorhinal cortex. We suggest that one consequence of these LPS- and IL-1β-induced changes is a compromise in glutamate release in dentate gyrus, which was coupled with the inhibition of LTP. The evidence is consistent with the idea that the LPS-induced impairment in LTP is mediated by IL-1β and is a consequence of activation of p38.

The proinflammatory cytokine, interleukin-1β (IL-1β),[1] exerts numerous effects in the central nervous system; among these effects is inhibition of long term potentiation (LTP) in the hippocampus (1–6). LTP in perforant path-granule cell synapses has been shown to be attenuated in aged and stressed rats (4, 5) and in rats that were treated intraperitoneally with lipopolysaccharide (LPS; Ref. 7) or intracerebroventricularly with IL-1β (4, 5); IL-1β concentration in hippocampus was increased in each of these experimental conditions, and a negative correlation between the ability of rats to sustain LTP and IL-1β concentration in hippocampus has been described previously (8, 9). The finding that the effect of LPS on LTP is mimicked by IL-1β, together with the finding that the effect of LPS on LTP was abrogated by inhibiting IL-1β converting enzyme (caspase-1; Ref. 7), suggested that IL-1β may mediate this effect of LPS.

Among the downstream effects of IL-1β is activation of the mitogen-activated protein kinases, c-Jun NH_2-terminal kinase (JNK), or stress-activated protein kinase (SAPK) and p38; this effect has been reported in several tissues, for example, IL-1β increases activity of p38 in Chinese hamster CCl39 (10) and HeLa (11) cells, while IL-1β-induced activation of JNK has been reported in human glomerular mesangial (12) and HeLa (11) cells. In the hippocampus, activation of JNK and p38 is coupled with elevated IL-1β concentrations in aged rats (13) and in rats treated intracerebroventricularly with IL-1β (6), and the LTP-associated increase in KCl-stimulated glutamate release is attenuated in both experimental conditions (6, 13). This is consistent with the observation that IL-1β inhibits transmitter release, for example release of acetylcholine (14) and glutamate (15) in hippocampal synaptosomes. This inhibitory effect of IL-1β on glutamate release may contribute to its effect on LTP, because, in dentate gyrus at least, maintenance of LTP seems to be partially dependent on an increase in glutamate release (16–19). Indeed it might be predicted that glutamate release is modulated by activity of p38, since the IL-1β-induced attenuation of LTP in dentate gyrus *in vitro* is overcome by the p38 inhibitor, SB203580 (20).

Evidence suggests that activation of the transcription factor, nuclear factor κB (NFκB), is stimulated by p38 activation (*e.g.* (21)), while the stimulatory effect of IL-1β on NFκB has been recognized for many years (see Ref. 22). The findings that NFκB is expressed in the brain (23), and that its activation is probably related to synaptic function (24), suggest that this may be part of the cascade of events triggered by IL-1β, and subsequently p38 phosphorylation, in hippocampus. NFκB is a three-subunit complex, consisting of an inhibitory subunit, IκB, a 50-kDa subunit (p50), and a 65-kDa subunit (p65), which exists in the cytoplasm. Upon activation, which involves phosphorylation of IκB, dissociation occurs and the dimer (p50/p65) translocates to the nucleus to stimulate gene transcription (see Ref. 25). NFκB activation in brain is triggered by a variety of stimuli, including oxidative stress, increased intracellular calcium concentration, glutamate, growth factors, and cytokines including IL-1β and tumor necrosis factor (see Refs. 22, 26, and 27). The evidence that its activation is increased in circumstances in which degenerative changes occur has been inter-

* This work was supported by the Health Research Board (Ireland) and the Higher Education Authority (Ireland). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 353-1-6083794/6081019; Fax: 353-1-6793545; E-mail: aikelly@tcd.ie.

[1] The abbreviations used are: IL, interleukin; LTP, long term potentiation; LPS, lipopolysaccharide; JNK, c-Jun NH_2-terminal kinase; NFκB, nuclear factor κB; PBS, phosphate-buffered saline; NGS, normal goat serum; PAO, phenylarsine oxide; DTT, dithiothreitol; PMSF, phenylmethylsulfonyl fluoride; DL-TBOA, DL-threo-β-benzyloxyaspartic acid; epsp, excitatory post-synaptic potential; ANOVA, analysis of variance.

preted by some as evidence of a pro-apoptotic role for NFκB (28–32). However powerful evidence from *in vitro* studies, and *in vivo* studies using knock-out mice, supports an anti-apoptotic role for NFκB (33–38).

This study was designed to investigate the consequences of the LPS-induced increase in IL-1β concentration in rat brain, focusing in particular on the possibility that activation of p38 and NFκB may be involved in the inhibition of LTP induced by LPS. We report that p38 activation in hippocampus was indeed one consequence of peripheral administration of LPS, that its activation was accompanied by increased activation of NFκB, and that inhibition of these changes by SB203580 and SN50 at least partially suppressed the inhibitory effects of LPS and IL-1β on LTP.

EXPERIMENTAL PROCEDURES

Animals—Groups of male Wistar rats (300–350 g) were housed in groups of 2–4 under a 12-h light schedule. Wild type and IL-1RI knock-out (IL-1R1$^{-/-}$) mice were housed in groups of 6 under a 12-h light schedule. Breeding pairs of IL-1R1$^{-/-}$ mice on a C57BL/6 background were obtained from Jackson Laboratories, and C57 mice were obtained from Harlan. Ambient temperature was controlled between 22 and 23 °C. Food and water were freely available. Wild type and IL-1R1$^{-/-}$ mice were randomly divided into two subgroups that received an intraperitoneal injection of saline (200 μl; 0.9%) or of *Escherichia coli* LPS (200 μl; 5 mg/ml). Mice were monitored for 3 h following injection after which they were killed by decapitation. Hippocampus was removed, dissected on ice, and cross-chopped into slices (350 × 350 μm) using a McIlwain tissue chopper. The time taken to prepare slices from the time of death was 2.5–3.5 min. All samples were frozen separately in 1 ml of Krebs solution (composition of Krebs solution in mM: NaCl, 136; KCl, 2.54; KH_2PO_4, 1.18; $MgSO_4 \cdot 7H_2O$, 1.18; $NaHCO_3$, 16; glucose, 10; $CaCl_2$, 1.13) containing 10% dimethyl sulfoxide according to the method of Haan and Bowen (39). For analysis, thawed slices of tissue were rinsed three times in fresh ice-cold Krebs solution and homogenized in either ice-cold sucrose (0.32 M) for preparation of P_2 (18) or in Krebs solution.

Staining for p-p38 and for p-IκB—Slices, prepared from hippocampus and entorhinal cortex, were equilibrated in oxygenated Krebs solution for 30 min at 30 °C and incubated in Krebs solution containing protease X (1 mg/ml), protease XIV (1 mg/ml), and DNase (1600 Kunitz) for 30 min at 30 °C. Washed slices were resuspended in 1 ml of prewarmed Dulbecco's modified essential medium containing DNase (1600 Kunitz), triturated with a glass Pasteur pipette and passed through a nylon mesh filter to remove tissue clumps. Aliquots (30 μl) were spun onto glass slides (600 rpm for 1 min), dipped in 100% methanol, and stored at room temperature. Cells were fixed in paraformaldehyde (4% in PBS) for 30 min at room temperature, washed with PBS, permeabilized with Triton-X100 (0.1% (v/v) in PBS for 5 min at room temperature, refixed in paraformaldehyde (4% in PBS) for 15 min at room temperature, and incubated in H_2O_2 (0.6% in PBS) for 5 min at room temperature to block endogenous peroxidases. Cells were washed in PBS, blocked in normal horse serum (1:20 (v/v) in PBS) for 30 min at room temperature, and incubated in phosphospecific anti-p38 antibody (1:100 in PBS; Santa Cruz) for 2 h at 37 °C. Cells were washed, incubated in biotinylated horse anti-mouse antibody (1:100; Vector Laboratories) for 30 min at room temperature, and reacted with a vectastain avidin biotin system for 15 min at room temperature to amplify the signal. Cells were incubated in diaminobenzidine chromogen solution containing H_2O_2 (0.1%) for 10 min at room temperature, rinsed in distilled H_2O; in some experiments cells were counterstained in methyl green. Cells were dehydrated through alcohol to xylene and mounted in DPX. Cells were counted, and p-p38 +ve and p-IκB +ve cells were expressed as a percentage of the total.

Because the physicochemical stresses involved in preparation of cytospun cells might lead to false positive results, we also prepared cryostat sections from saline and LPS-treated rats for assessment of p-p38 and p-IκB. In these experiments, 3 h after LPS injection (100 μg/kg, intraperitoneal) rats were killed, brains were removed whole, coated with OCT compound (Sakura Tissue-Tek), immersed in isopentane at −30 °C, and stored at −80 °C. Twenty-μm cryostat sections were prepared, mounted on gelatin-coated slides, air-dried for 30 min, and stored at −20 °C until used for immunohistochemical analysis.

For analysis of p-p38 and p-IκB, frozen cryostat sections were thawed at room temperature, washed in PBS, fixed in 4% paraformaldehyde, washed, permeabilized in 0.1% Triton in PBS, washed again, and refixed in 4% paraformaldehyde before non-reactive sites were blocked with 10% normal goat serum (NGS) in PBS. Sections were treated overnight in a humidified chamber at 4 °C with p-p38 (1:50 in 2.5% NGS) or p-IκB (1:100 in 2.5% NGS) antibody (Cell Signaling Technology). Sections were washed in PBS, incubated in the dark for 2 h at room temperature in fluorescein isothiocyanate-labeled goat anti-mouse IgG or IgM (1:200 in 2.5% NGS; BioSource), washed, and mounted with an aqueous mounting medium (Vector Laboratories) and sealed. Slides were viewed under a Zeiss fluorescence microscope at an excitation wavelength of 495 nm, and photomicrographs were taken at ×40 magnification.

Induction of LTP in Perforant Path-Granule Cell Synapses in Vivo—LTP was induced as described previously (18). Rats were injected intraperitoneally with urethane (1.5 g/kg), placed in a head holder in a stereotaxic frame, and injected intraperitoneally with saline or LPS (500 μg/kg); subgroups of LPS-treated rats were pretreated intracerebroventricularly, 5–10 min earlier, with saline (5 μl), SB203580 (50 μM; 5 μl), or SN50 (5 μl; 5 mg/ml). In a second series of experiments, rats were injected intracerebroventricularly with either IL-1β (5 μl; 3.5 ng/ml; human recombinant; 5×10^{-7} units/mg; The Biologic Response Modifiers Program, NCI, National Institutes of Health), IL-1β plus SB203580 (50 μM; 5 μl), IL-1β plus SN50 (5 μl; 5 mg/ml), or saline (5 μl). In a third series of experiments rats were treated intracerebroventricularly with saline, IL-1β, or IL-1β plus phenylarsine oxide (PAO; 5 μl; 100 μM). Animals were monitored for 3 h following LPS treatment and were then placed in the stereotaxic frame. In the case of IL-1β-treated rats, recording commenced 30 min following injection. In all cases, a window of skull was removed to allow placement of recording and stimulating electrodes in the molecular layer of the dentate gyrus (2.5 mm lateral and 3.9 mm posterior to bregma) and perforant path (angular bundle, 4.4 mm lateral to lambda), respectively. The depth of the electrodes was adjusted to obtain maximal responses in the cell body region. Stable base-line recordings were made for about 15 min, and sampling of the electrophysiological data then commenced. Test shocks at the rate of 1/30 s were delivered for 10 min prior to, and 40 min following, tetanic stimulation (three trains of stimuli; 250 Hz for 200 ms; intertrain interval, 30 s). At the end of the electrophysiological recording period, rats were killed by decapitation, the hippocampus was removed, and the tetanized and untetanized dentate gyri, as well as the hippocampus proper and entorhinal cortices, were dissected on ice, cross-chopped into slices, and frozen according to the method of Haan and Bowen (39).

Analysis of Caspase-1 Activity—Cleavage of the caspase-1 substrate (YVAD peptide, Alexis Corp.) to its fluorescent product was used as a measure of caspase-1 activity (7). Slices of tissue were washed, homogenized in lysis buffer (400 μl; 25 mM HEPES, 5 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 5 mM EDTA, 2 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml leupeptin, 10 μg/ml pepstatin, pH 7.4), subjected to four freeze-thaw cycles, and centrifuged at 15,000 rpm for 20 min at 4 °C. Samples of supernatant (90 μl) were added to YVAD peptide (500 μM; 10 μl) and incubated at 37 °C for 60 min. Incubation buffer (900 μl; 100 mM HEPES containing 10 mM DTT, pH 7.4) was added, and fluorescence was assessed (excitation, 400 nm; emission, 505 nm).

Analysis of IL-1β Concentration—IL-1β concentration in homogenate prepared from hippocampus was analyzed by enzyme-linked immunosorbent assay (13) (DuoSet; Genzyme Diagnostics). Antibody-coated (2.0 μg/ml final concentration, diluted in 0.1 M sodium carbonate buffer, pH 9.5; monoclonal hamster anti-mouse IL-1β antibody) 96-well plates were incubated overnight at 4 °C, washed four times with PBS containing 0.05% Tween 20, blocked for 2 h at 37 °C with 250 μl of blocking buffer (PBS, pH 7.3, 0.1 M with 4% bovine serum albumin), and incubated with IL-1β standards (100 μl; 0–1000 pg/ml) or samples for 1 h at 37 °C. Samples were incubated with secondary antibody (100 μl; final concentration 0.8 μg/ml in PBS containing 0.05% Tween 20 and 1% bovine serum albumin; biotinylated polyclonal rabbit anti-mouse IL-1β antibody) for 1 h at 37 °C, washed, and incubated in detection agent (100 μl; horseradish peroxidase-conjugated streptavidin; 1:1000 dilution in PBS containing 0.05% Tween 20 and 1% bovine serum albumin) and incubated for 15 min at 37 °C. 3,3′,5,5′-Tetramethylbenzidine (100 μl; Sigma) was added and incubated at room temperature, and absorbance was read at 450 nm within 30 min.

Analysis of p38 Phosphorylation—Samples of homogenate prepared from dentate gyrus of rats, hippocampus of mice, or entorhinal cortex of rats and mice were diluted to equalize for protein concentration (40), and p38 phosphorylation was assessed as described previously (13). Briefly, aliquots (10 μl, 1 mg/ml) were added to 10 μl of sample buffer (0.5 mM Tris-HCl, pH 6.8, 10% glycerol, 10% SDS, 5% β-mercaptoeth-

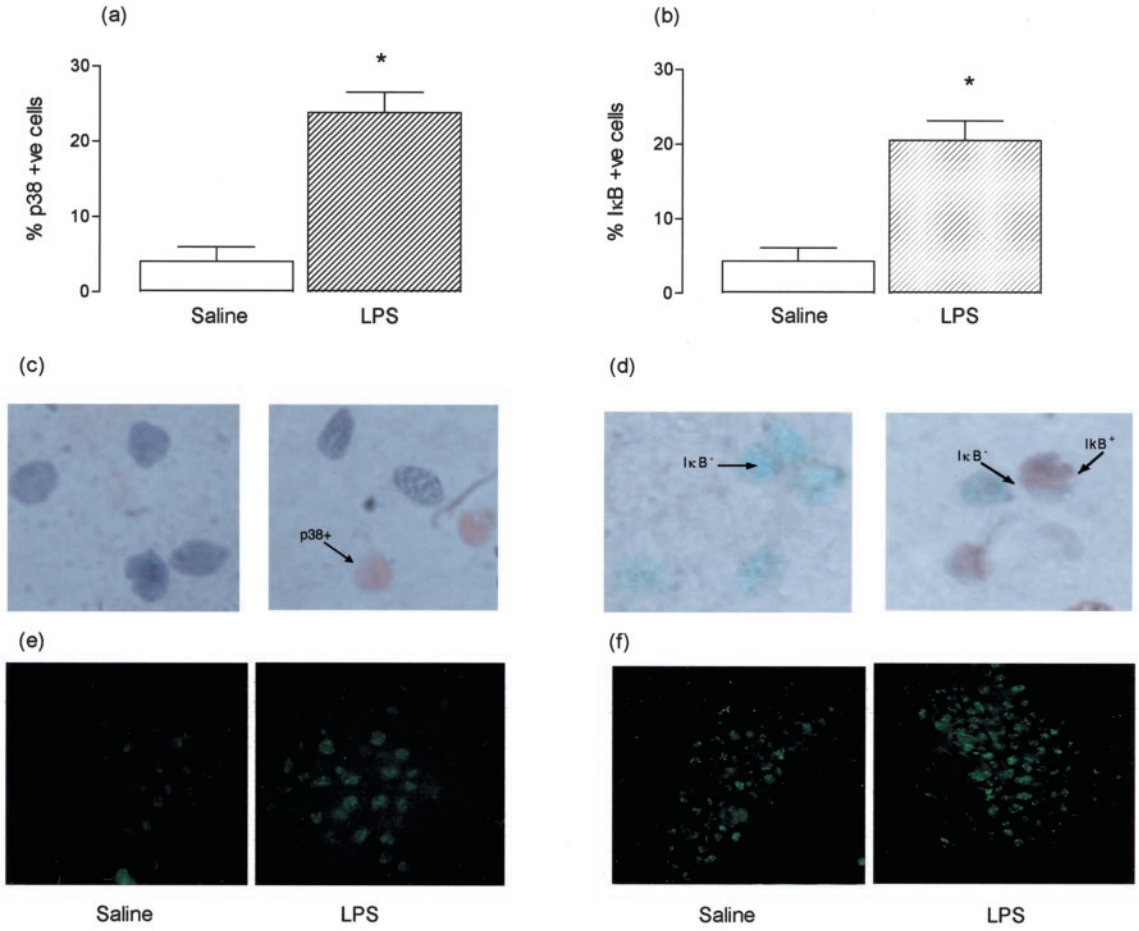

FIG. 1. **LPS increases phosphorylation of p38 and IκB.** Intraperitoneal injection of LPS significantly enhanced the number of hippocampal cells staining positively for phosphorylated p38 (p-p38; *a*) and phosphorylated IκB (p-IκB; $p < 0.05$; Student's *t* test for independent means) (*b*). The micrographs (*c* and *d*) provide examples of cells that were stained negatively and positively for both. Enhanced staining for p-p38 (*e*) and p-IκB (*f*) was also evident in cryostat sections prepared from LPS-treated, compared with saline-treated, rats. Photomicrographs were taken at ×40 magnification.

anol, 0.05% w/v bromphenol blue), boiled for 5 min, and loaded onto 10% SDS gels. Proteins were separated by application of 30 mA constant current for 25–30 min and transferred onto nitrocellulose strips (225 mA for 75 min). In the case of tissue prepared from rats, nitrocellulose strips were immunoblotted with an antibody that specifically targets phosphorylated p38 (Santa Cruz; 1:2000 in phosphate-buffered saline-Tween (0.1% Tween 20) containing 2% nonfat dried milk) for 2 h at room temperature. Immunoreactive bands were detected using peroxidase-conjugated anti-mouse IgM (Sigma) and ECL (Amersham Biosciences), exposed to film overnight, and processed using a Fuji x-ray processor. In the case of tissue prepared from mice, nitrocellulose strips were immunoblotted with an antibody that specifically targets phosphorylated p38 (Santa Cruz; 1:200 in Tris-buffered saline-Tween (0.1% Tween 20) containing 2% nonfat dried milk) for 3 h at room temperature. Immunoreactive bands were detected using peroxidase-conjugated anti-rabbit IgG (Amersham Biosciences) and SuperSignal West Dura (Pierce), exposed to film for 1 min, and processed using a Fuji x-ray processor. Quantitation of protein bands was achieved by densitometric analysis using two software packages, Grab It (Grab It Annotating Grabber 2.04.7, Synotics; UVP Ltd.) and Gelworks (Gelworks ID, Version 2.51; UVP Ltd.) for photography and densitometry, respectively. Gelworks provides a single value (in arbitrary units) representing the density of each blot, and the values presented here are means of data generated from six separate experiments.

Analysis of Glutamate Release—Glutamate release was assessed in the impure preparation, P_2, obtained from dentate gyrus; either freshly prepared tissue was used, or, alternatively, P_2 was prepared from frozen slices of dentate gyri that were obtained from rats in which electrophysiological recordings were made (see below). Glutamate release was also assessed in P_2 prepared from hippocampus of C57BL/6 IL-1R1$^{-/-}$ and wild type mice. In both cases, P_2 preparations were resuspended in oxygenated Krebs solution containing 2 mM $CaCl_2$ (18).

In a separate series of experiments, in an effort to assess the contribution, if any, of the glutamate-Na^+ exchanger, P_2 preparations were incubated for 5 min at 37 °C in the presence or absence of the non-transportable inhibitor DL-threo-β-benzyloxyaspartic acid (DL-TBOA; 100 μM).

In all cases, glutamate release was assessed as described previously (41, 42). Briefly, synaptosomal tissue was aliquotted onto Millipore filters (0.45 μm), rinsed under vacuum, and the filtrate was discarded. Synaptosomes were then incubated in 250 μl of oxygenated Krebs solution at 37 °C for 3 min, in the presence or absence of 40 mM KCl, and filtrate was collected and stored for analysis as described (43). In some experiments, synaptosomes were incubated at 37 °C for 15 min in Krebs solution containing IL-1β (1 ng/ml) in the presence or absence of the p38 inhibitor, SB203580 (50 μM). Triplicate samples (50 μl) or glutamate standards (50 μl; 25 nM to 1 μM prepared in 100 mM Na_2HPO_4 buffer, pH 8.0) were added to glutaraldehyde-coated 96-well plates, incubated for 60 min at 37 °C, and washed with 100 mM NaH_2PO_4 buffer. Ethanolamine (250 μl; 0.1 M in 100 mM Na_2HPO_4 buffer) was used to bind unreacted aldehydes, and donkey serum (200 μl; 3% in PBS-T) was added to block nonspecific binding. Samples were incubated overnight at 4 °C in the presence of antiglutamate antibody (raised in rabbit; 100 μl; 1:5000 in PBS-T; Sigma), washed, and reacted with secondary antibody (anti-rabbit horseradish peroxidase-linked secondary antibody; 100 μl; 1:10,000 in PBS-T; Amersham Biosciences) for 60 min at room temperature. 3,3′,5,5′-Tetramethylbenzidine liquid substrate was added as chromogen and incubation continued for exactly 60 min at room temperature, at which time the reaction was stopped by H_2SO_4 (4 M; 30 μl). Optical densities were determined at 450 nm using a multi-well plate reader, and values were calculated with reference to the standard curve, corrected for protein (40), and expressed as μmol of glutamate/mg of protein.

Activation of NFκB—Nuclear extracts were prepared from dissoci-

ated cells (above). The cell pellet was resuspended in hypotonic buffer (1 ml; composition in mM: HEPES, 10; $MgCl_2$, 1.5; KCl, 10; PMSF, 0.5; DTT, 0.5; pH 7.9) and centrifuged at 15,000 rpm at 4 °C for 5 min. The resulting pellet was resuspended in 20 μl of hypotonic buffer containing Nonidet P-40 (0.1%), incubated on ice for 10 min and centrifuged at 15,000 rpm at 4 °C for 10 min. To prepare the nuclear extract, the pellet was resuspended in buffer (15 μl; composition in mM: HEPES, 20; NaCl, 420; $MgCl_2$, 1.5; EDTA, 0.2; PMSF, 0.5; glycerol, 25% (v/v); pH 7.9), placed on ice for 30 min, and centrifuged 15,000 rpm at 4 °C for 10 min. Buffer (40 μl; composition in mM: HEPES, 10; KCl, 50; EDTA, 0.2; PMSF, 0.5; DTT, 0.5; glycerol, 20% (v/v); pH 7.9) was added to the resulting supernatant and equalized for protein concentrations.

A 22-base pair oligonucleotide containing the NFκB consensus sequence (Promega) was labeled with [^{32}P]ATP (10 mCi/mmol) by incubation with T4 polynucleotide kinase (Promega). Nuclear extracts were incubated with labeled oligonucleotide (20,000 cpm) in buffer (composition in mM: Tris-HCl, 10; NaCl, 100; EDTA, 1; DTT, 5; glycerol, 4% (v/v); nuclease-free bovine serum albumin, 0.1 mg/ml (w/v); pH 7.5) containing poly(dI.dC; 2 μg, Pharmacia Biotech) as a nonspecific competitor for 30 min at room temperature. Samples were electrophoresed on 5% native polyacrylamide gels, which were dried under vacuum and exposed to x-ray film for 5–7 days.

Statistical Analysis—A one-way analysis of variance was performed to determine whether there were significant differences between conditions. When this analysis indicated significance (at the 0.05 level), post hoc Student's Newmann-Keuls test analysis was used to determine which conditions were significantly different from each other. The Student's *t* test was used to establish statistical significance in some cases, for example when analysis was performed on aliquots of the same tissue incubated in the presence/absence of IL-1β. The use of the Student's *t* test for paired values was also appropriate when analyses were performed on tissue prepared from untetanized and tetanized tissue from the same rat.

RESULTS

Intraperitoneal injection of LPS resulted in a significant increase in the number of hippocampal cells that stained positively with an anti-phosphospecific p38 antibody. Analysis of the mean data obtained from six LPS-treated and six saline-treated rats indicated that 24% of the cells in the LPS-treated tissue stained positively compared with 4% of the cells prepared from saline-treated tissue ($p < 0.01$; Student's *t* test for independent means; Fig. 1, *a* and *c*). The effect of LPS on p38 phosphorylation in hippocampus was confirmed by immunohistochemical analysis of brain sections (Fig. 1*e*). Phosphorylation of IκB, which precedes NFκB activation, is a consequence of p38 activation and was also assessed in hippocampal cells prepared from LPS-treated and saline-treated rats. Fig. 1, *b* and *d*, show that LPS treatment was associated with increased phosphorylation of IκB and analysis of the cell counts from the two treatment groups revealed that a significantly greater proportion of hippocampal cells prepared from LPS-treated rats (compared with saline-treated rats) stained positively for activated IκB ($p < 0.01$; Student's *t* test for independent means). The effect of LPS on IκB phosphorylation in hippocampus was confirmed by immunohistochemical analysis of brain sections (Fig. 1*f*).

Fig. 2 shows that intraperitoneal injection of LPS inhibited both the early and later phases of LTP; thus the mean percentage increase in population epsp slope in the 2 min immediately following tetanic stimulation (compared with that in the 5 min immediately before the tetanus) was 109.8 ± 4.82% in LPS-treated rats compared with 163.3 ± 3.47% in saline-treated rats ($p < 0.01$; ANOVA). The mean percentage changes in the last 5 min of the experiment were 90.9 ± 0.93% and 141.9 ± 1.14% in LPS-treated and saline-treated rats, respectively ($p < 0.01$; ANOVA; $n = 6$). In view of the finding that LPS activated p38 and NFκB, we considered that the inhibitory action on LTP may be a consequence of these changes. In an effort to address this question, the effects of the p38 inhibitor, SB203580, and the NFκB inhibitor, SN50, on LPS-induced inhibition of LTP were assessed. Intracerebroventricular injection of SB203580

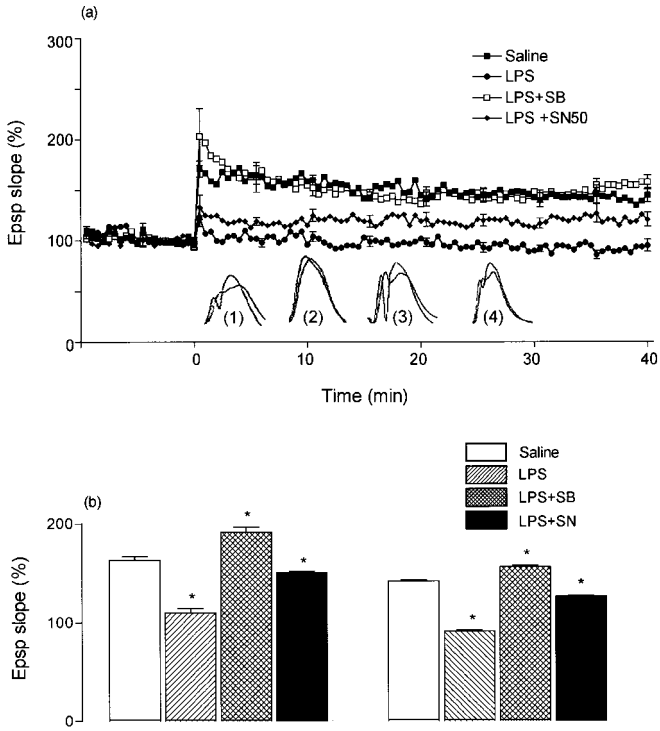

FIG. 2. **LPS inhibits LTP.** *a*, intraperitoneal injection of LPS blocked tetanus-induced LTP in perforant path-granule cell synapses, but this effect was suppressed by intracerebroventricular injection of SB203580 or SN50. The data are expressed as the mean percentage change in population epsp slope (compared with the epsp slope in the 5 min immediately prior to tetanic stimulation). Values are means ± S.E. of six rats in each treatment group. These values were obtained by calculating epsp slopes collected in response to stimuli delivered at 30-s intervals, expressing as a percentage change relative to pretetanus values and taking a mean of these values from all animals in that treatment group. *b* and *c*, the mean percentage changes in epsp slopes in the 2 min (*b*) and 35–40 min (*c*) following tetanic stimulation indicate the significant inhibitory effect of LPS ($p < 0.01$; ANOVA) on the early and later components of LTP. Pretreatment with SB203580 reversed the effect of LPS, although the values were significantly greater than those observed in saline-treated rats. Similarly SN50 blocked the LPS-induced inhibition, although in this case, values were significantly decreased compared with saline-treated rats ($p < 0.01$; ANOVA in each case). Superimposed sample pre- and post-tetanus EPSP traces are shown for each treatment group (saline (*trace 1*), LPS (*trace 2*), LPS + SB203580 (*trace 3*); LPS + SN50 (*trace 4*)).

prevented the LPS-induced attenuation in LTP such that the mean percentage increases in epsp slope in the 2 min immediately after tetanic stimulation and in the last 5 min of the experiment were 191.3 ± 5.23% and 155.3 ± 0.65%, respectively (Fig. 2, *b* and *c*; $n = 6$). These values were both significantly greater than the corresponding values in the saline-treated group ($p < 0.01$; ANOVA). Pretreatment of rats with the NFκB inhibitor, SN50, attenuated the LPS-induced effect on LTP; the mean percentage changes in the epsp slope in the 2 min immediately after tetanic stimulation and in the last 5 min of the experiment were 126.8 ± 2.09% and 120.9 ± 0.65%, respectively (Fig. 2, *b* and *c*; $n = 6$). These values were significantly greater than the corresponding values in the LPS-treated group but significantly less than those in the saline-treated group ($p < 0.01$; ANOVA).

Previous work had indicated that the action of LPS may be mediated through IL-1β, and consistent with this is the finding that caspase-1 activity was significantly enhanced in hippocampal tissue prepared from rats that were injected intraperitoneally with LPS compared with saline-injected rats ($p < 0.05$; Student's *t* test for independent means; Fig. 3*a*). This increase in enzyme activity was accompanied by an increase in

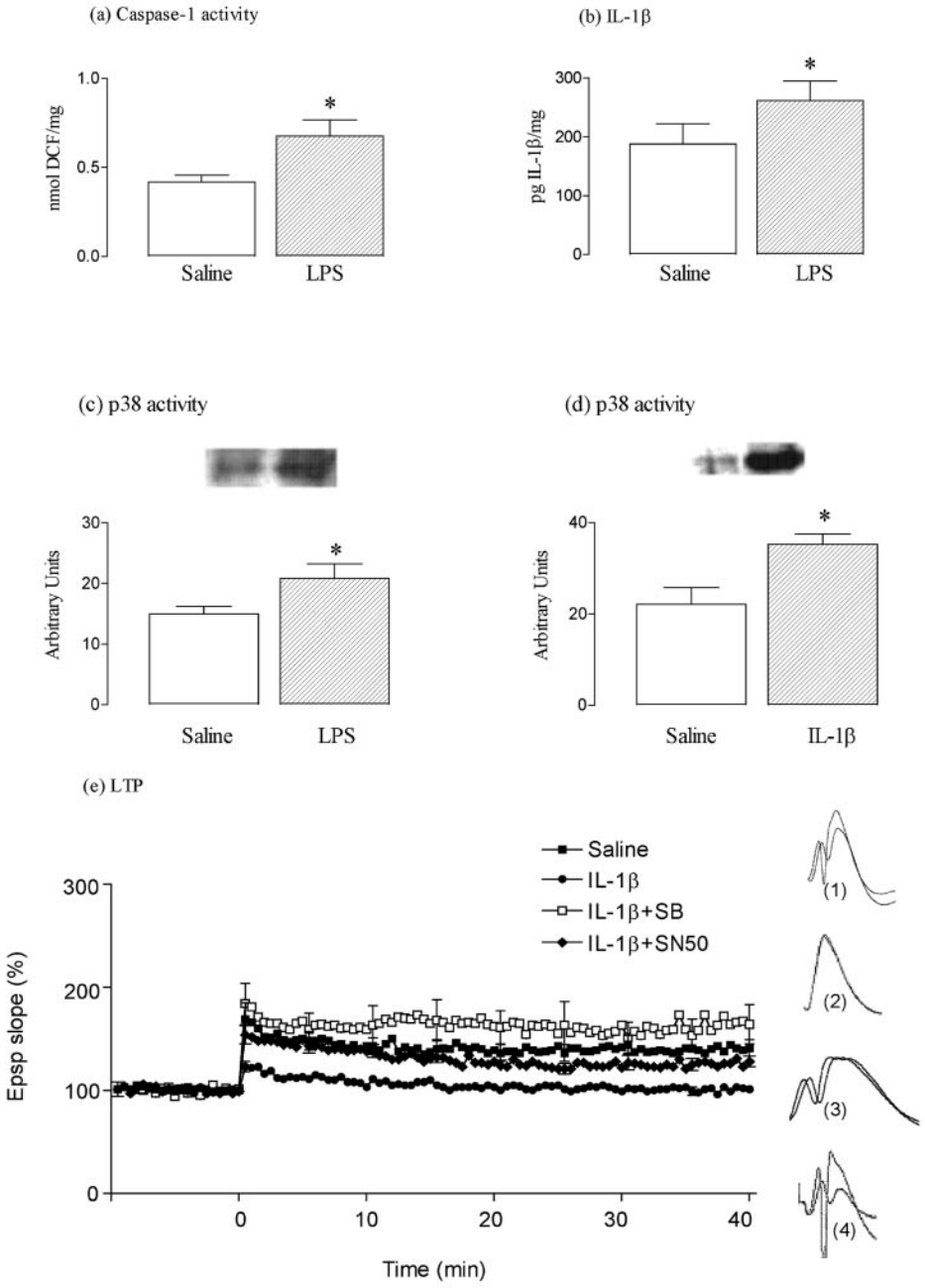

FIG. 3. **IL-1β may mediate the effects of LPS by stimulating p38 activation.** Caspase-1 activity (*a*) and IL-1β concentration (*b*) were significantly enhanced by LPS ($p < 0.05$; Student's *t* test for paired means; $n = 6$ in each case). Both LPS (*c*) and IL-1β (*d*) enhanced activity of p38 as shown by the sample immunoblot (compare *right-hand lanes* with *left-hand lanes*) and by analysis of the mean data obtained from densitometric analysis LPS ($p < 0.05$; Student's *t* test for paired means; $n = 6$ in each case). *e*, intracerebroventricular injection of IL-1β blocked tetanus-induced LTP in perforant path-granule cell synapses, but this effect was suppressed by intracerebroventricular injection of SB203580 or SN50. The data are expressed as the mean percentage change in population epsp slope (compared with the epsp slope in the 5 min immediately prior to tetanic stimulation; see legend to Fig. 2). Values are means ± S.E. of six rats in each treatment group. Superimposed sample pre- and post-tetanus EPSP traces are shown for each treatment group (saline (*trace 1*), IL-1β (*trace 2*), IL-1β + SB203580 (*trace 3*), and IL-1β + SN50 (*trace 4*)).

the concentration of IL-1β, so that cytokine concentration was significantly increased in hippocampus of LPS-, compared with saline-, treated rats ($p < 0.05$; Student's *t* test for independent means; Fig. 3*b*). In parallel with these changes we observed an LPS-associated increase in phosphorylation of p38 in synaptosomes prepared from dentate gyrus; one sample immunoblot is presented, as well as the mean data derived from densitometric analysis of the six individual experiments, which indicate a significant effect of LPS treatment (Fig. 3*c*; $p < 0.05$; Student's *t* test for independent means). Fig. 3*d*, which shows one sample immunoblot and mean data derived from densitometric analysis, indicates that the LPS-induced effect on p38 phosphorylation was mimicked by intracerebroventricular injection of IL-1β ($p < 0.05$ in each case; $n = 6$; Student's *t* test for paired means).

If IL-1β mediates the effect of LPS, then IL-1β should mimic the effects of LPS, and any procedure that abrogates the effect of LPS should also abrogate the effect of IL-1β. Fig. 3*e* shows that LTP was inhibited by IL-1β and that this effect was suppressed by SB203580. The mean percentage increases (±S.E.) in the 2 min immediately following tetanic stimulation (compared with the mean value in the 5 min immediately prior to the tetanus) were 161.8 ± 3.75%, 121.0 ± 1.68%, and 175.5 ± 4.21% in the groups injected with saline, IL-1β, and IL-1β + SB203580, respectively. The corresponding values in the last 5 min of the experiment were 140.2% ± 0.75, 100.4 ± 0.73%, and 163.8 ± 1.97%. Thus the effect of IL-1β mimicked that of LPS, and the inhibitory effect of SB203580 on IL-1β-induced attenuation of LTP mirrored its similar effect on LPS. These findings suggest that the LPS-induced inhibition of LTP is mediated by activation of p38. Fig. 3*e* also indicates that the inhibitory effect of IL-1β on LTP was partially suppressed by SN50; thus the mean percentage changes in the epsp slope in the 2 min immediately after tetanic stimulation and in the last 5 min of the experiment in rats treated with IL-1β and SN50 were 150.5 ± 1.5% and 126.3 ± 0.76%, respectively. On the basis of these observations it can be proposed that activation of NFκB plays some part in the inhibition of LTP by IL-1β. To

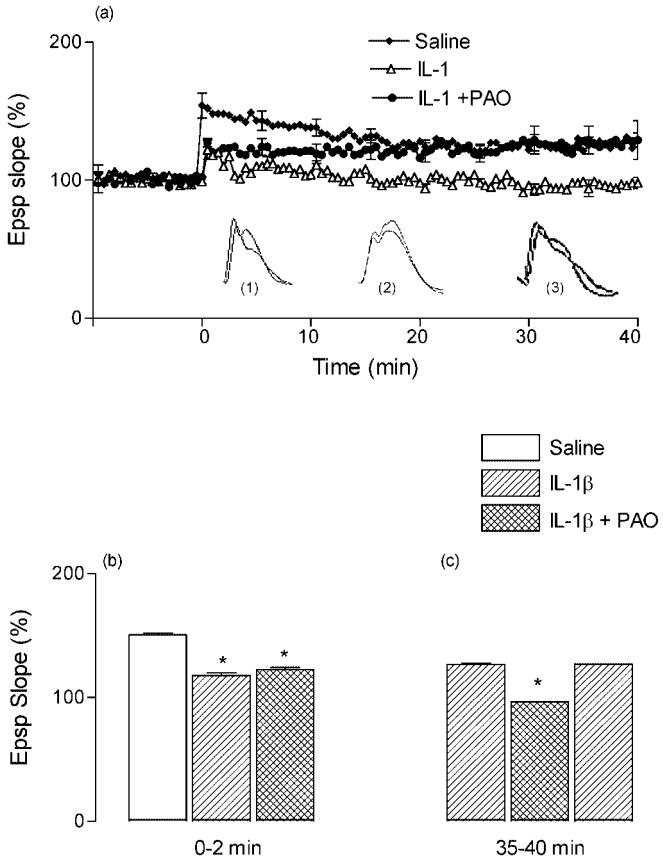

FIG. 4. **PAO abrogates the inhibitory effect of IL-1β on LTP.** Intracerebroventricular injection of IL-1β blocked tetanus-induced LTP in perforant path-granule cell synapses, but this effect was suppressed by intracerebroventricular injection of PAO. The data are expressed as the mean percentage change in population epsp slope (compared with the epsp slope in the 5 min immediately prior to tetanic stimulation; see legend to Fig. 2). Values are means ± S.E. of six rats in each treatment group. Superimposed sample pre- and post-tetanus EPSP traces are shown for each treatment group (saline (*trace 1*), IL-1β (*trace 2*), IL-1β + PAO (*trace 3*)).

provide further support for this proposal we investigated the effect of another, albeit nonspecific inhibitor of NFκB, PAO, on the inhibition of LTP induced by IL-1β. Fig. 4 indicates that while IL-1β depressed LTP as previously described, pretreatment of rats with PAO attenuated the action of IL-1β. The mean percentage change in population epsp slope in the 2 min immediately following tetanic stimulation was significantly decreased in IL-1β-treated, compared with saline-treated, rats (117.5 + 2.63% compared with 150.5 ± 1.50%; $p < 0.01$; ANOVA; Fig. 4*b*). This difference persisted for the duration of the experiment so that the value in the last 5 min of the experiment was significantly lower in IL-1β-treated (96.7 ± 0.53%), compared with saline-treated (126.6 ± 0.87%) rats ($p < 0.01$; ANOVA; Fig. 4*c*). Pretreatment with PAO did not reverse the IL-1β-induced change in epsp slope in the 2 min following tetanic stimulation (122.5 ± 1.66%; $p > 0.05$) but reversed the IL-1β-associated deficit in the last 5 min of the experiment, so that the mean percentage change in population epsp slope at this time was similar in rats treated with saline (126.6 ± 0.87%) and those treated with IL-1β + PAO (126.9 ± 0.68%).

Glutamate release was assessed in synaptosomes prepared from untetanized and tetanized dentate gyrus prepared from rats that were treated with saline, IL-1β, IL-1β + SB203580, and IL-1β + SN50. The data obtained from these analyses are shown in Fig. 5 and demonstrate that addition of KCl (40 mM) to synaptosomes prepared from untetanized dentate gyrus of saline-treated rats significantly increased glutamate release ($p < 0.05$; Student's *t* test for paired means), but that the increase was more marked in synaptosomes prepared from tetanized dentate gyrus ($p < 0.01$; Student's *t* test for paired means). KCl failed to increase glutamate release in untetanized and tetanized tissue prepared from IL-1β-treated rats, but in tissue prepared from rats treated with both IL-1β and SB203580, addition of KCl induced a significant increase in glutamate release in synaptosomes prepared from both untetanized ($p < 0.05$; Student's *t* test for paired means) and tetanized ($p < 0.01$; Student's *t* test for paired means) dentate gyrus. The data also indicate that SN50 partially reversed the inhibitory effect of IL-1β on KCl-stimulated release so that depolarizsation significantly increased glutamate release in synaptosomes prepared from untetanized ($p < 0.05$; Student's *t* test for paired means), but not tetanized ($p > 0.05$) dentate gyrus. In an effort to establish whether the glutamate-Na^+ exchanger contributed to the glutamate concentration in these samples, we analyzed release in the presence and absence of the inhibitor, DL-TBOA. The data obtained from 10 separate experiments indicated that DL-TBOA slightly increased release in some, and slightly decreased release in other, experiments; the overall effect was to slightly, but not significantly ($p > 0.05$), enhance unstimulated release (1.77 μmol/mg ± 0.30 *versus* 2.30 μmol/mg ± 0.38 in control- and DL-TBOA-treated samples, respectively) and KCl-stimulated release (2.64 μmol/mg ± 0.029 and 3.50 μmol/mg ± 0.48). We therefore conclude that the glutamate-Na^+ exchanger does not contribute significantly to the concentration of glutamate in our samples.

These findings suggest that IL-1β-induced activation of p38 and NFκB exerted a negative effect on glutamate release, and since the cell bodies of the synapses at which this effect occurs are located in the entorhinal cortex, we argued that evidence of activation of p38 and NFκB may also extend to this area. Fig. 6, *a* and *c*, show that treatment with LPS and IL-1β both led to a marked increase in the number of entorhinal cortical cells that stained positively for p38 ($p < 0.05$ in both cases; Student's *t* test for independent means; see Fig. 6*b* for examples of p38 +ve and −ve cells, which were counted to yield the mean data). This figure also shows examples of fluorescently labeled cells stained positively with anti-p-p38 antibody in cryostat sections prepared from LPS-treated and IL-1β-treated rats; these contrast with the lack of staining in sections prepared from saline-treated controls. These changes were coupled with activation of NFκB; two examples of this activation induced by LPS (Fig. 7*a*) and IL-1β (Fig. 7*b*) are shown and similar data were obtained in three and five additional experiments in the case of LPS and IL-1β, respectively. In an effort to establish whether p38 activation was responsible for IL-1β-induced activation of NFκB, we assessed activation of the transcription factor *in vitro* and report that this measure was markedly increased when tissue was incubated in the presence of IL-1β alone, but that activation was suppressed in tissue which was incubated in the presence of IL-1β and SB203580. One example of this is shown in Fig. 7*c*; similar data were obtained in five additional experiments.

In an effort to consolidate the data that suggested that IL-1β mediated the effect of LPS, we investigated LPS-induced changes in p-p38 and glutamate release in tissue prepared from C57BL/6 IL-1R1$^{-/-}$ and wild type mice. The data presented in Fig. 8 indicate that LPS significantly increased p38 activation in hippocampus and entorhinal cortex prepared from wild type mice (*, $p < 0.05$; **, $p < 0.01$; ANOVA) but not C57BL/6 IL-1R1$^{-/-}$ mice. Although mean p38 phosphorylation was slightly greater in tissue prepared from C57BL/6 IL-1R1$^{-/-}$ mice, this difference was not statistically significant.

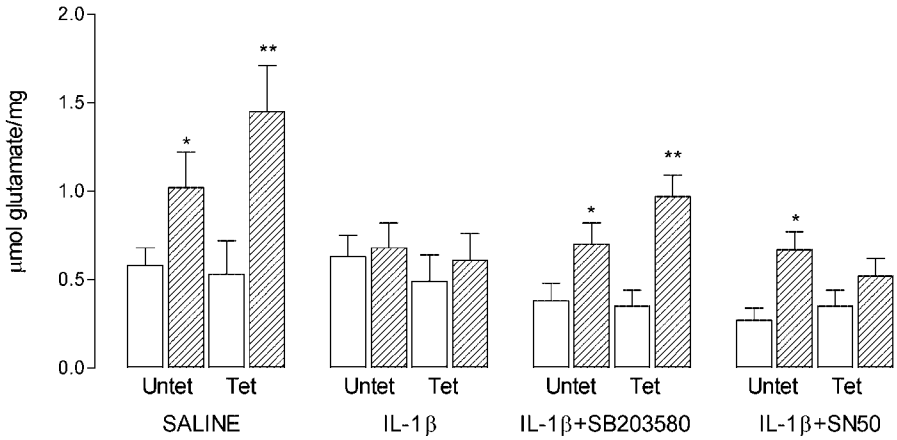

FIG. 5. **IL-1β-induced inhibition of glutamate release is abrogated by SB203580 and SN50.** Glutamate release was assessed in synaptosomes prepared from dentate gyrus of rats treated with saline or IL-1β ($n = 12$), IL-1β + SB203580 ($n = 6$), or IL-1β + SN50 ($n = 6$). Analysis revealed that addition of KCl (40 mM) to untetanized tissue prepared from saline-treated rats significantly enhanced release ($p < 0.05$; Student's t test for paired means) and that this effect was further enhanced in synaptosomes prepared from tetanized dentate gyrus ($p < 0.01$; Student's t test for paired means). KCl-stimulated release was inhibited in untetanized and tetanized tissue prepared from IL-1β-treated rats, but SB203580 reversed this inhibitory effect; thus incubation in the presence of KCl resulted in an increase in release in both untetanized and tetanized tissue (*, $p < 0.05$ and **, $p < 0.01$; Student's t test for paired means). SN50 also reversed the inhibitory effect of KCl in untetanized tissue ($p < 0.05$) but failed to restore the enhanced release in tissue prepared from tetanized dentate gyrus).

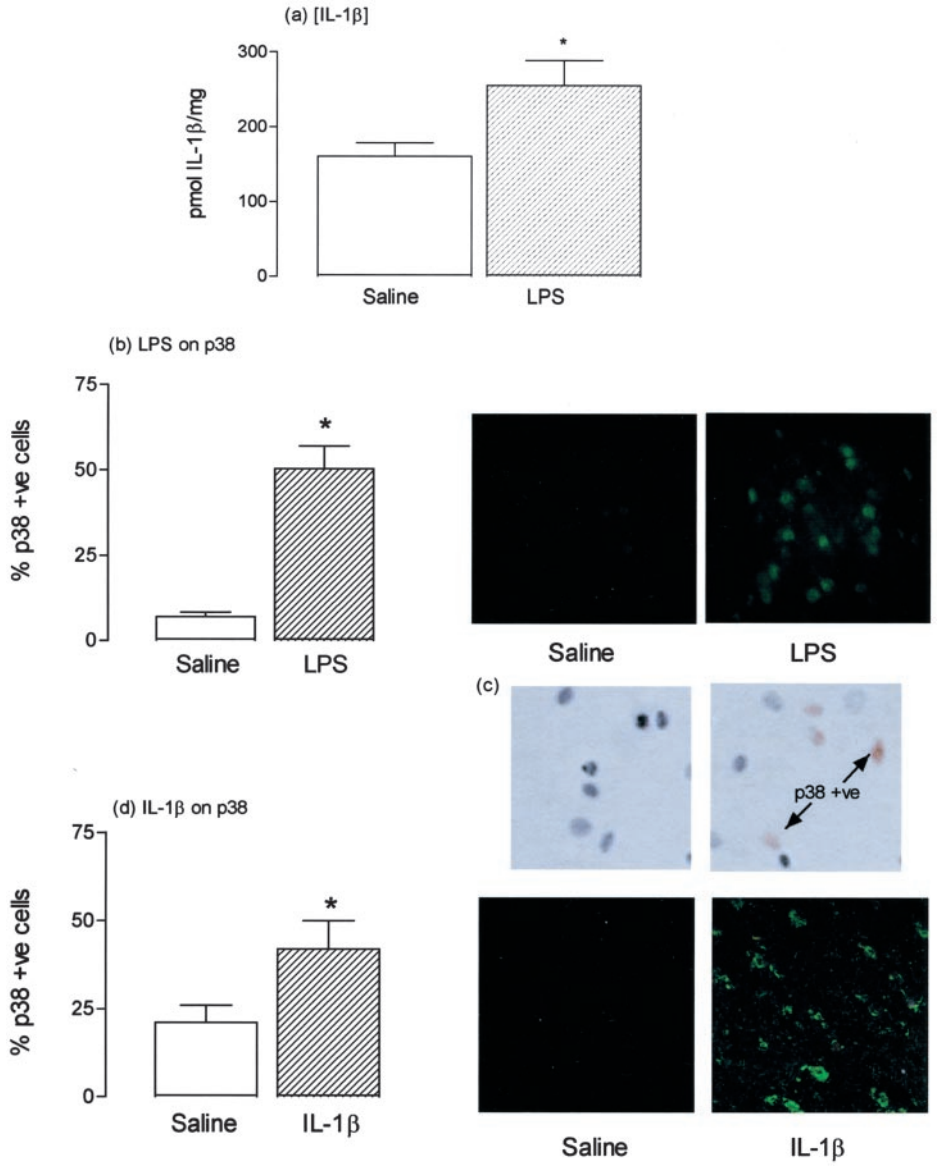

FIG. 6. **LPS and IL-1β increase p38 activation.** Intraperitoneal injection of LPS (*a*) and intracerebroventricular injection of IL-1β (*c*) resulted in a significant enhancement in the number of entorhinal cortical cells staining positively for phosphorylated p38 (*b*; $p < 0.01$; Student's t test for independent means). Examples of negatively and positively stained cells (which where those counted to provide the mean data) are shown in *b*. Examples of fluorescent staining in cryostat sections prepared from LPS- and IL-1β-treated rats are also presented.

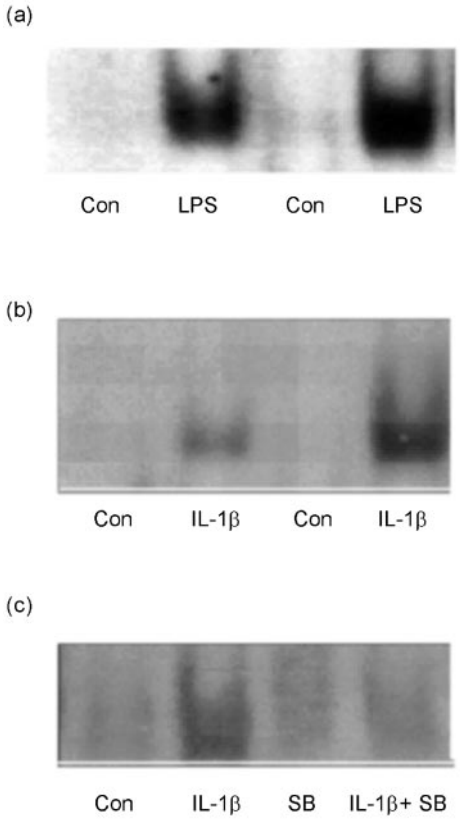

FIG. 7. **LPS and IL-1β increase NFκB activation.** Intraperitoneal injection of LPS (*a*) and intracerebroventricular injection of IL-1β (*b*) resulted in a significant enhancement in NFκB activation in entorhinal cortical cells; replicate examples are shown. *c*, analysis of NFκB activation *in vitro* revealed that the stimulatory effect of IL-1β was abrogated by SB203580.

Fig. 8*c* indicates that LPS significantly decreased KCl-stimulated glutamate release (minus unstimulated release) in tissue prepared from wild type mice, but not C57BL/6 IL-1R1$^{-/-}$ mice, which further supports the view that IL-1β mediates the effect of LPS.

DISCUSSION

The objective of this study was to identify the cellular changes induced by peripheral administration of LPS that might lead to inhibition of LTP in perforant path-granule cell synapses. The evidence presented suggests that LPS-induced phosphorylation of p38, mediated by IL-1β, triggers a cascade of changes in the cells, which is followed by deterioration of cell function. We propose that these events are likely to explain the LPS-induced suppression of LTP in perforant path-granule cell synapses.

Intraperitoneal injection of LPS led to a sharp increase in the number of hippocampal cells which stained positively for phosphorylated p38, and this was closely coupled by an increase in the number of cells staining positively for phosphorylated NFκB. At least in some cell types, one consequence of increased p38 phosphorylation is activation of NFκB (21). Consistent with this is the observation that the ischemia-reperfusion injury in myocardial tissue, associated with activation of NFκB, was inhibited by SB203580 (44). The evidence presented is consistent with the idea that IL-1β mediates the cellular effects induced by LPS in hippocampus as observed in other tissues, for example neutrophils (45) and lymphocytes (46). Thus intraperitoneal injection of LPS increased caspase-1 activity in hippocampus and IL-1β concentration in both hippocampus and entorhinal cortex, which is consistent with our finding that

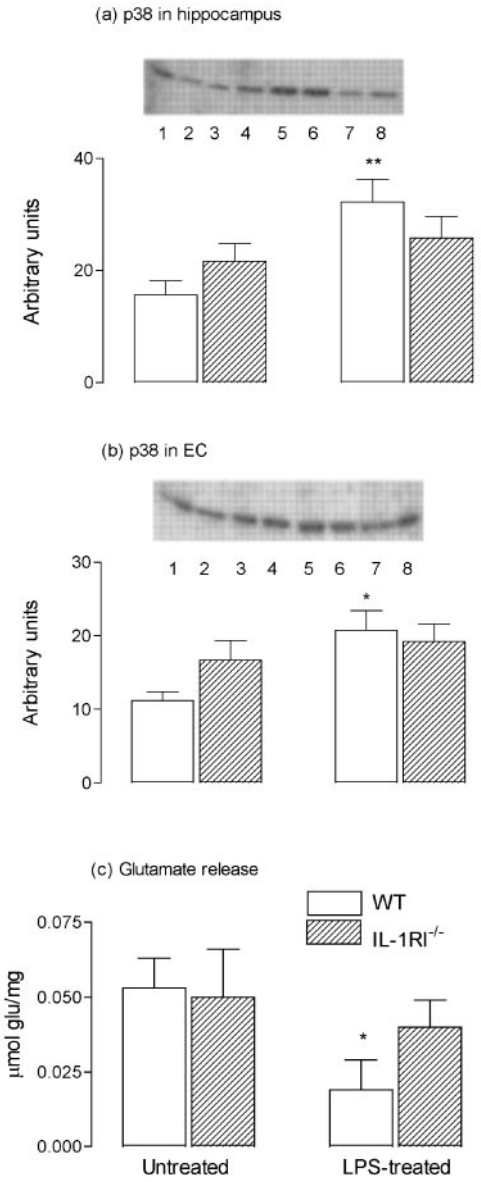

FIG. 8. **C57BL/6 IL-1R1$^{-/-}$ mice are resistant to LPS-induced effects on p38 activation and glutamate release.** *a* and *b*, densitometric analysis revealed that phosphorylation of p38 in hippocampus (*a*) and entorhinal cortex (*b*) of wild type mice was significantly increased following LPS treatment (**, $p < 0.01$ and *, $p < 0.05$ in the case of hippocampus and EC respectively; ANOVA; *lanes 5* and *6*) compared with saline treatment (*lanes 1* and *2*) as shown by the sample immunoblot. LPS treatment of C57BL/6 IL-1R1$^{-/-}$ mice (*lanes 7* and *8*) failed to affect p38 phosphorylation (compare with saline treatment; *lanes 3* and *4*). Values are means ± S.E. of six mice in each treatment group. *c*, KCl-stimulated glutamate release in hippocampus of wild type mice was significantly decreased following LPS treatment ($p < 0.05$; ANOVA) compared with saline treatment, but this LPS-induced effect was absent in C57BL/6 IL-1R1$^{-/-}$ mice. Values are means ± S.E. of six mice in each treatment group.

several effects of LPS on synaptic activity are inhibited by blocking the activity of caspase-1 (7). The present data also indicate that the stimulatory effect of LPS on p38 activity was mimicked by IL-1β, supporting the results of other experiments which concluded that the LPS-induced effect on p38 was mediated by IL-1β (see Ref. 47). Analysis of the effect of LPS on activation of p38 and glutamate release in C57BL/6 IL-1R1$^{-/-}$ mice indicated a marked attenuation in response compared with that observed in wild type mice; this finding strengthens the evidence indicating that IL-1β mediates the LPS-induced effect in neuronal tissue.

In further support of this view is the finding that both LPS and IL-1β blocked LTP in dentate gyrus, which confirms previous observations (4–7). We have not addressed the question of whether the effect of these agents is on the induction or maintenance phase of LTP. However, Coogan and O'Connor (67) have reported that IL-1β had no effect on LTP in dentate gyrus *in vitro* when applied post-induction of LTP, indicating that the inhibitory effect of IL-1β on LTP may be specifically on the induction phase. To assess the argument that the effect of LPS and IL-1β was mediated through activation of p38 and NFκB, we examined the ability of the p38 inhibitor, SB203580, and the NFκB inhibitors, SN50 and PAO, to reverse the suppression of LTP induced by both agents. The data indicate that pretreatment with SB203580 fully restored the ability of LPS-injected and IL-1β-injected rats to sustain LTP; indeed we observed that the mean change in epsp slope was greater in rats treated with IL-1β + SB203580 compared with those treated with saline. These data are strikingly similar to those observed *in vitro* (20). In contrast to the effectiveness of SB203580, pretreatment with SN50 only partially reversed the inhibitory effects of LPS and IL-1β on LTP; an additional striking difference between the effect of SB203580 and SN50 was the observation that the immediate response to tetanic stimulation was markedly attenuated in rats treated with SN50 + LPS and SN50 + IL-1β. Similarly, PAO failed to reverse the early inhibitory effect of IL-1β, although mean epsp slope at the end of the 40-min experiment was similar in rats treated with saline and those treated with PAO + IL-1β.

Data from a number of earlier studies indicated that enhanced IL-1β concentration in hippocampus is coupled, not only with impaired LTP, but also with compromised glutamate release; examples include tissue prepared from aged rats (13, 41, 42) and rats treated with IL-1β or LPS (6, 7). To address the possibility that this compromise in glutamate release was a consequence of activation of p38 and NFκB, we analyzed glutamate release in synaptosomes of dentate gyrus prepared from rats treated with IL-1β with or without SB203580 and SN50. We found that glutamate release was attenuated in IL-1β-treated rats, confirming earlier findings (6), and that this was abrogated by both SB203580 and SN50. Reflecting the changes observed in the electrophysiological experiments, we found that SB203580 was more effective in abrogating the inhibition induced by IL-1β than was SN50. The parallel shift in potentiation (or inhibition of potentiation) of the synaptic response and glutamate release in the different treatment groups in this study support the hypothesis that LTP in dentate gyrus is tightly coupled with an increase in glutamate release at perforant path-granule cell synapses (17, 18, 41, 42).

On the basis of these findings, it seems reasonable to conclude that the negative impact of IL-1β on LTP is a consequence of increased p38 phosphorylation and activation of NFκB. It has been reported that NFκB is highly expressed in the hippocampus and that expression of mRNA for p50, p65, and IκB increased in granule cells following synaptic activity (24). It was reported that expression of the p65 subunit was stimulated to a greater degree than the p50 subunit, but that changes occurred with both low and high frequency stimulation and both ipsilaterally and contralaterally. We have compared NFκB activation in cells prepared from dentate gyrus of control rats and rats that sustained LTP in perforant path-granule cell synapses and found no consistent change: roughly one-third showing a slight increase in activation, a third showing a slight decrease, and the remainder showing no change. Therefore, in the time frame of the present experiments, delivery of a high frequency tetanus does not appear to affect NFκB activation.

It might be argued that any change in glutamate release at perforant path-granule cell synapses will be accompanied by, and perhaps may be caused by, a change in the cell bodies of the synapses in the entorhinal cortex, and consistent with this is the observation that the LTP-associated increase in glutamate release is accompanied by increased protein synthesis (48) and increased synthesis of the synaptic vesicle proteins, synapsin and synaptophysin (48, 49). Here we found that both LPS treatment and IL-1β treatment markedly increased the number of entorhinal cortical cells staining positively for phosphorylated p38. In addition to increased activation of p38, both treatments were associated with increased activation of NFκB, which supports the view that activation of p38 and NFκB occur in concert and that both are activated by IL-1β in neuronal tissue. Consistent with this is the observation that the IL-1β-induced activation of NFκB *in vitro* could be suppressed by SB203580.

The question of the cellular consequences resulting from increased activation of p38 and NFκB, which are responsible for the attenuation of glutamate release and LTP, have not been addressed in this study. However it has been shown that p38 activation leads to growth arrest or even cell death in some cells (50, 51), for example following cell exposure to various stressors including oxidative stress (11, 12, 52). Similarly increased p38 activation has been coupled with degenerative changes in the nervous system (53–55), and this has been carefully documented in cortical cultures (*e.g.* Refs. 56 and 57). It is significant that increased p38 activity has been shown to signal apoptosis in several tissues, including hippocampus (58, 59). In striking similarity, increased NFκB has also been coupled with degenerative changes, for example, following seizure activity (28, 60, 61), *N*-methyl-D-aspartate receptor-induced damage (31, 32), and ischemic insults (29, 62, 63). In addition NFκB activation is increased in the aged brain (64) and in pathological conditions like Alzheimer's disease and Parkinsons' disease (29, 30, 65, 66). However, evidence from *in vitro* studies, and *in vivo* studies using knock-out mice, supports a protective role for NFκB. Thus it has been found that the antiapoptotic effect of tumor necrosis factor (33) and ceramide (34) in cultured cells involved activation of NFκB, while inhibition of NFκB induced apoptosis in PC12 cells (36). Similarly, NFκB has been shown to protect hippocampal neurons from apoptosis induced by oxidative stress (35) or by hypoxia (37). Resolution of these apparently conflicting data awaits further study, and although the data presented here suggest that activation of p38 and NFκB are associated with compromised synaptic function, the mechanism by which this occurs remains to be elucidated.

The hypothesis that IL-1β plays a significant role in mediating the effects of LPS is supported by the findings that LPS injection results in increased activity of caspase-1 in hippocampus and increased concentration of IL-1β in hippocampus and entorhinal cortex. In addition, IL-1β mimics the effects of LPS, both lead to increased activation of p38 and NFκB in entorhinal cortex and hippocampus, and both treatments exert an inhibitory effect of LTP. The observations that SB203580 and SN50 ameliorate the inhibitory effects of LPS and IL-1β provide support for the view that their activation has a negative impact on synaptic function. On the basis of these data, we suggest that IL-1β-induced activation of p38 markedly affects synaptic function at perforant path-granule cell synapses and results in attenuation of LTP.

REFERENCES

1. Bellinger, F. P., Madamba, S., and Siggins, G. R. (1993) *Brain Res.* **628,** 227–234
2. Katsuki, H., Nakai, S., Hirai, Y., Akaji, K., Kiso, Y., and, Satoh, M. (1990) *Eur. J. Pharmacol.* **181,** 323–326
3. Cunningham, A. J., Murray, C. A., O'Neill, L. A. J., Lynch, M. A., and O'Connor, J. J. (1996) *Neurosci. Lett.* **203,** 1–4

4. Murray, C., and Lynch, M. A. (1998) *J. Biol. Chem.* **273,** 12161–12168
5. Murray, C., and Lynch, M. A. (1998) *J. Neurosci.* **18,** 2974–2981
6. Vereker, E., O'Donnell, E., and Lynch, M. A. (2000a) *J. Neurosci.* **20,** 6811–6819
7. Vereker, E., Campbell, V., Roche, E., McEntee, E., and Lynch, M. A. (2000b) *J. Biol. Chem.*, **275,** 26252–26528
8. Lynch, M. A. (1998) *Prog. Neurobiol.* **56,** 1–19
9. Lynch, M. A. (1999) *Mol. Psychiatry* **4,** 15–18
10. Guay, J., Lambert, H., Gingras-Breton, G., Lavoie, J. N., Huot, J., and Landry, J. (1997) *J. Cell Sci.*, **110,** 357–368
11. Raingeaud, J., Gutpa, S., Rogers, J. S., Dickens, M., Han, J., Ulevitch, R. J., and Davis, R. J. (1995) *J. Biol. Chem.*, **270,** 7420–7426
12. Uciechowski, P., Saklatvala, J., von der Ohe, J., Resch, K., Szamel, M., and Kracht, M. (1996) *FEBS Lett.* **394,** 273–278
13. O'Donnell, E., Vereker, E., and Lynch, M. A. (2000) *Eur. J. Neurosci.* **12,** 345–352
14. Rada, P., Mark, G. P., Vitek, M. P., Manago, R. M., Blume, A. J., Beer, B., and Hoebel, B. G. (1991) *Brain Res.* **550,** 287–290
15. Murray, C. A., McGahon, B., McBennett, S., and Lynch, M. (1997) *Neurobiol. Aging* **18,** 343–348
16. Bliss, T. V. P., and Collingridge, G. L. (1993) *Nature* **361,** 31–39
17. Canevari, L., Richter-Levin, G., and Bliss, T. V. P. (1994) *Brain Res.* **667,** 115–117
18. McGahon, B., and Lynch, M. A. (1996) *Neuroscience* **72,** 847–855
19. McGahon, B., Clements, M. P., and Lynch, M. A. (1997) *Neuroscience* **81,** 9–16
20. Coogan, A. N., O'Neill, L. A. J., and O'Connor, J. J. (1999) *Neuroscience*, **93,** 57–69
21. Carter, A. B., Knudtson, K. L., Monick, M. M., and Hunninghake, G. W. (1999) *J. Biol. Chem.*, **274,** 30858–30863
22. Baeuerle, P. A., and Henkel, T. (1994) *Annu. Rev. Immunol.*, **14,** 649–668
23. Kaltschmidt, C., Kaltschmidt, B., and Baeuerle, P. A. (1993) *Mech. Dev.* **43,** 135–147
24. Meberg, P. J., Kinney, W. R., Valcourt, E. G., and Routtenberg, A. (1996) *Mol. Brain Res.* **38,** 179–190
25. Mattson, M. P., Culmsee, C., Yu, Z., and Camandola, S. (2000) *J. Neurochem.* **74,** 443–456
26. O'Neill, L. A. J., and Kaltschmidt, C. (1997) *Trends Neurosci.* **20,** 252–258
27. Pahl, H. L. (1999) *Oncogene* **18,** 6853–6866
28. Grilli, M., Pizzi, M., Memo, M., and Spano, P. (1996) *Science* **274,** 1383–1385
29. Clemens, J. A., Stephenson, D. T., Dixon, E. P., Smalstig, E. B., Mincy, R. E., Rash, K. S., and Little, S. P. (1997) *Mol. Brain Res.* **48,** 187–196
30. Kaltschmidt, B., Uherek, M., Volk, B., Baeuerle, P. A., and Kaltschmidt, C. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 2642–2647
31. Qin, Z.-H., Wang, Y., Nakai, M., Chase, T. N. (1998) *Mol. Pharmacol.* **53,** 33–42
32. Qin, Z.-H., Chen, R.-W., Wang, Y., Nakai, M., Chuang, D.-M., and Chase, T. N. (1999) *J. Neurosci.* **19,** 4023–4033
33. Barger, S. W., Horster, D., Furukawa, K., Goodman, Y., Krieglstein, J., and Mattson, M. P. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 9328–9332
34. Goodman, Y., and Mattson, M. P. (1996) *J. Neurochem.* **66,** 869–872
35. Mattson, M. P., Goodman, Y., Luo, H., Fu, W., and Furukawa, K. (1997) *J. Neurosci. Res.* **49,** 681–697
36. Taglialatela, G., Robinson, R., and Perez-Polo, J. R. (1997) *J. Neurosci. Res.* **47,** 155–162
37. Tamatani, M., Mitsuda, N., Matsuzaki, H., Okado, H., Miyake, S., Vitek, M. P., Yamaguchi, A., and Tohyama, M. (2000) *J. Neurochem.* **75,** 683–693
38. Yu, Z., Zhou, D., Bruce-Keller, A. J., Kindy, M. S., and Mattson, M. P. (1999) *J. Neurosci.* **19,** 8856–8865
39. Haan, E. A., and Bowen, D. M. (1981) *J. Neurochem.* **37,** 243–246
40. Bradford, M. M. (1976) *Anal. Biochem.* **72,** 248–254
41. McGahon, B. M., Murray, C. A., Horrobin, D. F., and Lynch, M. A. (1999) *Neurobiol. Aging* **20,** 643–653
42. McGahon, B. M., Martin, D. S. D., Horrobin, D. F., and Lynch, M. A. (1999) *Neuroscience* **94,** 305–314
43. Ordronneau, P., Abdullah, L., and Petruse P. (1991) *J. Immunol. Methods* **142,** 169–176
44. Maulik, N., Sato, M., Price, B. D., and Das, D. K. (1998) *FEBS Lett.* **429,** 365–369
45. Nick, J. A., Avdi, N. J., Gerwins, P., Jognson, G. L., and Worthen, G. S. (1996) *J. Immunol.* **156,** 4867–4875
46. Lee, M., Yea, S. S., and Jeon, Y. J. (2000) *Int. J. Immunopharmacol.* **22,** 615–621
47. Benveniste, E. N. (1997) in *Immunology of the Nervous System* (Keane, R. W., and Hickey, W. F., eds) pp. 419–459, Oxford University Press, UK
48. Kelly, A., Mullany, P. M., and Lynch, M. A. (2000) *Hippocampus* **10,** 431–437
49. Mullany, P., and Lynch, M. A. (1997) *Neuropharmacology* **36,** 973–980
50. Park, D. S., Stefanis, L., Yan, C. Y. I., Farinelli, S. E., and Greene, L. A. (1996) *J. Biol. Chem.* **271,** 21898–21905
51. Maroney, A. C., Glicksman, M. A., Basma, A. N., Walton, K. M., Knight, E., Jr., Murphy, C. A., Bartlett, B. A., Finn, J. P., Angeles, T., Matsuda, Y., Neff, N. T., and Dionne, C. A. (1998) *J. Neurosci.* **18,** 104–111
52. Junger, W. G., Hoyt, D. B., Hamreus, M., Liu, F. C., Herdon-Remelius, C., Junger, W., and Altman, A. (1997) *J. Trauma Inj. Infect. Crit. Care* **42,** 437–445
53. Luo, Y., Umegaki, H., Wang, X., Abe, R., and Roth, G. S. (1998) *J. Biol. Chem.* **273,** 3756–3764
54. Mielke, K., Brecht, S., Dorst, A., and Herdegen, T. (1999) *Neuroscience* **91,** 471–483
55. Mielke, K., and Herdegen, T. (2000) *Prog. Neurobiol.* **61,** 45–60
56. Behrens, M. M., Strasser, U., Koh, J.-Y., Gwag, B. J., and Choi, D. W. (1999) *Neuroscience* **94,** 917–927
57. Namgung, U., and Xia, Z. (2000) *J. Neurosci.* **20,** 6442–6451
58. Ozawa, H., Shioda, S., Dohi, K., Matsumoto, H., Mizushima, H., Zhou, C. J., Funahashi, H., Nakai, Y., Nakajo, S., and Matsumoto, K. (1999) *Neurosci. Lett.* **262,** 57–60
59. Takagi, Y., Nozaki, K., Sugino, T., Hattori, I., and Hashimoto, N. (2000) *Neurosci. Lett.* **294,** 117–120
60. Rong, Y., and Baudry, M. (1996) *J. Neurochem.* **67,** 662–668
61. Matsuoka, Y., Kitamura, Y., Okazaki, M., Terai, K., and Taniguchi, T. (1999) *Exp. Brain Res.* **124,** 215–222
62. Salminen, A., Liu, P. K., and Hsu, C. Y. (1995) *Biochem. Biophys. Res. Commun.* **212,** 939–944
63. Zhang, S., Tobaru, T., Zivin, J. A., and Shackelford, D. A. (1998) *Mol. Brain Res.* **63,** 121–132
64. Toliver-Kinsky, T., Papaconstantinou, J., and Perez-Polo, J. R. (1997) *J. Neurosci. Res.* **48,** 580–587
65. Terai, K., Walker, D. G., McGeer, E. G., and McGeer, P. L. (1997) *Brain Res.* **769,** 385–390
66. Hunot, S., Brugg, B., Ricard, D., Michel, P. P., Muriel, M. P., Ruberg, M., Faucheux, B. A., Agid, Y., and Hirsch, E. C. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 7531–7536
67. Coogan, A. N., and O'Connor, J. J. (1997) *Neuroreport* **8,** 2107–2110