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The Anti-inflammatory Cytokine, Interleukin (IL)-10, Blocks the Inhibitory Effect of IL-1β on Long Term Potentiation

A ROLE FOR JNK*

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Several effects of the proinflammatory cytokine, interleukin-1β (IL-1β), have been described in the central nervous system, and one area of the brain where marked changes have been reported is the hippocampus. Among these changes are an IL-1β-induced inhibition of long term potentiation (LTP) in perforant path-granule cell synapses and an attenuation of glutamate release in synaptosomes prepared from the hippocampus. Evidence suggests that, at least in circulating cells, the anti-inflammatory cytokine, IL-10, antagonizes certain effects of IL-1. We investigated the effect of IL-10 on IL-1β-induced inhibition of LTP and glutamate release. The evidence presented indicates that IL-1β stimulates the stress-activated protein kinase, c-Jun-activated protein kinase (JNK), and IL-1 receptor-associated kinase, which may explain its inhibitory effect on release and LTP, and that IL-10 reversed the IL-1β-induced stimulation of JNK activity and inhibition of release and LTP. We observed that IL-10 abrogated the stimulatory effect of IL-1β on superoxide dismutase activity and reactive oxygen species production, whereas the H_2O_2-induced inhibition of LTP was also blocked by IL-10. We present evidence that suggests that the action of IL-10 may be mediated by its ability to induce shedding of the IL-1 type I receptor.

Interleukin-1β (IL-1β)[1] is a proinflammatory cytokine that is released from antigen-presenting cells during infection or inflammation, and although its effects were originally considered to be confined to the immune system, it is now known to exert profound effects in the central nervous system. These effects include modulation of thermoregulation, sleep, and appetite, which are perhaps consistent with the relatively high expression of the signal-generating IL-1 type 1 receptors (IL-1R1) in hypothalamus (1–5). However, IL-1β also inhibits transmitter release (6, 7) and calcium channel activity (7, 8) in the hippocampus, and it has been shown to inhibit long term potentiation (LTP) in CA1, CA3, and dentate gyrus *in vitro* (9–11) and in dentate gyrus *in vivo* (12–15); these effects are consistent with the high distribution of IL-1R1 in hippocampus (1–5). The inhibitory effects of IL-1β in hippocampus have been linked with stimulation of the stress-activated kinases, p38 and JNK (14, 16), which have also been shown to be activated by IL-1β in other cells (17–20). Evidence suggests that activation of IL-1 receptor-activated kinase (IRAK) is closely linked with JNK activation (21). Among the documented consequences of enhanced activity of JNK and/or p38 in some cells are growth arrest and deterioration of cell function or even cell death (22, 23).

In contrast to the proinflammatory effects of IL-1β, IL-10 has been shown to possess anti-inflammatory properties. Like IL-1β, IL-10 was originally identified as a product of certain cells of the immune system, *i.e.* T helper cells, B cells, monocytes, and macrophages (24, 25), although more recently it has been suggested that IL-10 is produced by cells in the hypothalamus and pituitary (26). IL-10 is co-released with IL-1β following injection of lipopolysaccharide (LPS (27, 28)), but it has been shown to inhibit the production of IL-1β and TNFα in LPS-activated macrophages (24). IL-10 has also been shown to reverse the IL-1-induced fever that follows LPS injection (29), whereas IL-1β induces slow wave sleep (30), IL-10 reduces sleep (31). At the level of the hippocampus, it has been shown that recovery following traumatic brain injury was improved by treatment with IL-10, and this was associated with decreased concentration of IL-1 in hippocampus (32).

The evidence therefore indicates that IL-10 inhibits certain actions of IL-1β, in some cases by inhibiting IL-1β production and/or release. In an effort to examine this question further, we set out to establish whether IL-10 might antagonize the inhibitory effect of IL-1β on synaptic function in the hippocampus. The data indicate that IL-10 abrogates the IL-1β-induced inhibition of glutamate release and LTP and its stimulatory effect on JNK. We propose that this action of IL-10 may be mediated by its ability to prevent reactive oxygen species production by IL-1β.

EXPERIMENTAL PROCEDURES

Animals—Male Wistar rats (BioResources Unit, Trinity College, Dublin, Ireland) were used in these experiments. Animals were housed in groups of 4–6 under a 12-h light schedule; ambient temperature was

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[1] The abbreviations used are: IL-1β, interleukin-1β; IL-1R1, IL-1 type 1 receptor; LTP, long term potentiation; IRAK, IL-1 receptor-activated kinase; JNK, c-Jun NH_2-terminal kinase; LPS, lipopolysaccharide; TNF, tumor necrosis factor; VIP, vasoactive intestinal protein; ERK, extracellular signal-regulated kinase; ANOVA, analysis of variance; epsp, excitatory postsynaptic potential.

controlled between 22 and 23 °C, and rats were maintained under veterinary supervision.

Phosphorylation of Mitogen-activated Protein Kinases and IRAK—The activities of ERK (33) and JNK (16) were analyzed in P_2 preparations obtained from dentate gyrus. Tissue samples were equalized for protein concentration (34) and diluted so that the same concentration of protein (1 mg/ml) was loaded onto each lane. In experiments in which the effect of vasoactive intestinal protein (VIP) was assessed, P_2 preparations were made in the presence of VIP (1 μM) allowing incorporation of the peptide into synaptosomes before membranes resealed. In other experiments, samples (which were/were not prepared in the presence of VIP) were preincubated for 15 min in IL-1β (1 ng/ml), IL-10 (10 ng/ml), H_2O_2 (200 μM), or a combination of some of these agents; in all circumstances, control samples were incubated in vehicle (Krebs solution containing 1.8 mM $CaCl_2$) only. In a separate series of experiments, synaptosomes were prepared from dentate gyrus of rats that were injected intracerebroventricularly with saline or IL-1β (3.5 ng/ml) or H_2O_2 (200 μM). Aliquots (10 μl, 1 mg/ml) were added to sample buffer (10 μl; Tris-HCl, 0.5 mM, pH 6.8; glycerol 10%; SDS, 10%; β-mercaptoethanol, 5%; bromphenol blue, 0.05% w/v), boiled for 5 min, and loaded onto gels (10% SDS for ERK; 12% for JNK). Proteins were separated by application of 30 mA constant current for 25–30 min, transferred onto nitrocellulose strips (225 mA for 75 min), and immunoblotted with the appropriate antibody. To assess ERK activity, proteins were immunoblotted overnight at 4 °C with an antibody specific for the phosphorylated form of ERK (Promega; 1:4,000 in phosphate-buffered saline/Tween (0.1% Tween 20; PBS-T) containing 2% non-fat dried milk). To assess JNK activity, proteins were immunoblotted with an antibody that specifically targets phosphorylated JNK (Santa Cruz Biotechnology; 1:2,000 in PBS-T (0.1% Tween 20) containing 2% non-fat dried milk) for 2 h at room temperature. To assess IRAK, proteins were immunoblotted with a rabbit polyclonal anti-IRAK-1 antibody (1:4,000 Tris-buffered saline/Tween (0.1% Tween 20 containing 0.1% bovine serum albumin) for 2 h at room temperature. In all cases, nitrocellulose strips were washed and incubated for 2 h at room temperature with secondary antibody (horseradish peroxidase-linked anti-rabbit antibody; 1:10,000 dilution (Amersham Pharmacia Biotech) in the case of ERK, horseradish peroxidase-linked anti-rabbit antibody; 1:1,000 dilution (Amersham Pharmacia Biotech) in the case of IRAK, and peroxidase-linked anti-mouse IgG; 1:2,000 dilution (Sigma) in the case of JNK). Protein complexes were visualized by ECL detection (Amersham Pharmacia Biotech) in the case of ERK and JNK and Supersignal (Pierce) in the case of IRAK. Immunoblots were exposed to film for 3–4 h in the case of ERK, overnight in the case of JNK, and 10 s in the case of IRAK and processed using a Fuji x-ray processor. Protein bands were quantitated by densitometric analysis.

Release of Glutamate—The impure synaptosomal preparation, P_2, was prepared as described previously (35) and resuspended in oxygenated Krebs solution containing 2 mM $CaCl_2$. Synaptosomes were preincubated for 15 min at 37 °C in oxygenated Krebs solution containing 2 mM $CaCl_2$ or Krebs solution to which 1 ng/ml IL-1β, 10 ng/ml IL-10, or both were added. In some experiments, synaptosomes were prepared in the presence of VIP (1 μM) as described above and were subsequently incubated with or without IL-1β (1 ng/ml) or H_2O_2 (200 μM). Tissue samples were aliquoted onto Millipore filters (0.45 mm), rinsed under vacuum, and then incubated in 250 μl of oxygenated Krebs solution at 37 °C for 3 min in the presence or absence of 40 mM KCl. The filtrate was collected and stored at −80 °C for later analysis (36). Triplicate samples (50 μl) or glutamate standards (50 μl; 50 nM to 10 μM prepared in 100 mM Na_2HPO_4 buffer, pH 8.0) were added to glutaraldehyde-coated 96-well plates, incubated, and washed. Ethanolamine (250 μl; 0.1 M in 100 mM Na_2HPO_4 buffer) was used to bind any unreacted aldehydes, and donkey serum was used to block nonspecific binding. Antiglutamate antibody (raised in rabbit; 100 μl; 1:5,000 in PBS-T; Sigma) was added, incubated, washed, and reacted with secondary antibody (anti-rabbit horseradish peroxidase-linked antibody; 100 μl; 1:10,000 in PBS-T; Amersham Pharmacia Biotech). 3,3′,5,5′-Tetramethylbenzidine liquid substrate was added as chromogen; samples were incubated for exactly 60 min at room temperature, and H_2SO_4 (4 M; 50 μl) was added to stop the reaction. Optical densities were determined at 450 nm using a multiwell plate reader, and values were calculated with reference to the standard curve, corrected for protein (34) and expressed as μmol of glutamate/mg of protein.

Analysis of IL-1R1 Expression—Samples (dentate gyrus synaptosomes), which were preincubated for 20 min at 37 °C in IL-1β (1 ng/ml), IL-10 (10 ng/ml), or both, were assessed for IL-1R1 expression by gel electrophoresis and immunoblotting. Following incubation, samples underwent one freeze-thaw cycle and were centrifuged (10,000 × *g* for 10 min). The supernatant was used to assess soluble IL-1R1, and the pellet, which was resuspended in Krebs solution containing 2 mM $CaCl_2$, was used to assess membrane-associated IL-1R1. In both cases, samples were equalized for protein concentration, and then proteins were separated by application of 30 mA constant current for 25–30 min, transferred onto nitrocellulose strips (225 mA for 75 min), and blocked overnight at 4 °C in PBS-T containing 6% non-fat dried milk. After appropriate washing (5 times 10-min washes in PBS-T), membranes were incubated in the primary antibody (rabbit anti-rat IL-1R1 IgG (Santa Cruz Biotechnology; 1:1,000 in PBS-T containing 2% non-fat dried milk)) for 45 min at room temperature and 45 min at 37 °C, washed (4 times 10-min washes in PBS-T), incubated in the secondary antibody (horseradish peroxidase-linked anti-rabbit, 1:2,000 in PBS-T containing 2% non-fat milk) for 45 min at room temperature and 45 min at 37 °C, and washed. Protein complexes were visualized by ECL detection (Amersham Pharmacia Biotech) by exposing immunoblots to film for overnight at 4 °C and processed using a Fuji x-ray processor. Protein bands were quantitated by densitometric analysis.

Induction of LTP in Vivo—Rats were anesthetized by intraperitoneal injection of urethane (1.5 g/kg intraperitoneal); the absence of a pedal reflex was considered to be an indicator of deep anesthesia. LTP was induced unilaterally in perforant path-granule cell synapses as described previously (12, 13). Briefly, a bipolar stimulating electrode and an unpopular recording were stereotaxically positioned in the perforant path (4.4 mm lateral to λ) and dorsal cell body region of the dentate gyrus (2.5 mm lateral and 3.9 mm posterior to Bregma), respectively. Rats were injected intracerebroventricularly (2.5 mm posterior, and 0.5 mm lateral, to Bregma) with saline, IL-1β (3.5 ng/ml) alone, or together with IL-10 (35 ng/ml or 1 μg/ml) or with H_2O_2 (200 μM) alone, or together with IL-10 (1 μg/ml); injection volume was 5 μl in all cases. Test shocks were given at 30-s intervals and recorded for 10 min before and 40 min after tetanic stimulation (3 trains of stimuli; 250 Hz for 200 ms; 30 s intertrain interval). Tetanic stimulation was delivered 40 min after injection.

Analysis of Reactive Oxygen Species Formation—The formation of reactive oxygen species was assessed by analyzing formation of the highly fluorescent 2′,7-dichlorofluorescein from the non-fluorescent probe, 2′7′-dichlorofluorescein diacetate (Molecular Probes (37)). The synaptosomal pellet, P_2, was prepared from hippocampus and resuspended in 1 ml of ice-cold 40 mM Tris buffer, pH 7.4. Samples were incubated at 37 °C for 15 min in the presence of 2′7′-dichlorofluorescein diacetate (10 μl; final concentration 5 μM; from a stock solution of 500 μM in methanol) to which IL-1β (1 μg/ml) and/or IL-10 (10 ng/ml) was added. To terminate the reaction, the dye-loaded synaptosomes were centrifuged at 13,000 × *g* for 8 min. The pellet was resuspended in 3 ml of ice-cold 40 mM Tris buffer, pH 7.4. Fluorescence was monitored at a constant temperature of 37 °C immediately before stimulation with IL-1β (1 ng/ml) and 15 min post-stimulation, at 488 nm excitation (bandwidth 5 nm), and 525 nm emission (bandwidth 20 nm). Reactive oxygen species formation was quantified from a standard curve of 2′,7-dichlorofluorescein in methanol (range 0.05 to 1 μM). Protein concentration was determined (34), and the results were expressed as nmol/mg protein/min.

Analysis of Superoxide Dismutase Activity—Superoxide dismutase activity was determined according to the method described previously (38). Briefly, hippocampal slices were homogenized in Krebs solution containing $CaCl_2$ and centrifuged at 15,000 for 10 min. Aliquots (800 μl) of incubation buffer (50 mM potassium buffer (pH 7.8) containing 1.8 mM xanthine, 2.24 mM nitro blue tetrazolium, 40 units of catalase, 7 μl/ml xanthine oxidase, and 1.33 mM diethylenetriaminepentaacetic acid) were added to samples of supernatant (100 μl) at different dilutions (1:2, 1:5, 1:10, 1:20, 1:50, and 1:100) and analyzed by UV spectroscopy at 560 nm. In some experiments, slices were incubated for 30 min at 37 °C in IL-1β (100 pg/ml) in the presence/absence of IL-10 (10 ng/ml) to analyze the effect of the cytokines on superoxide dismutase activity. Enzyme activity was assessed as the rate of reduction of nitro blue tetrazolium, which was inhibited with increasing concentrations of protein. One unit of activity was defined as the amount of protein necessary to decrease the rate of the reduction of nitro blue tetrazolium by 50%.

RESULTS

Fig. 1*A* shows that IL-1β (1 ng/ml) increased JNK activity as indicated by an increase in the phosphorylated form of JNK;

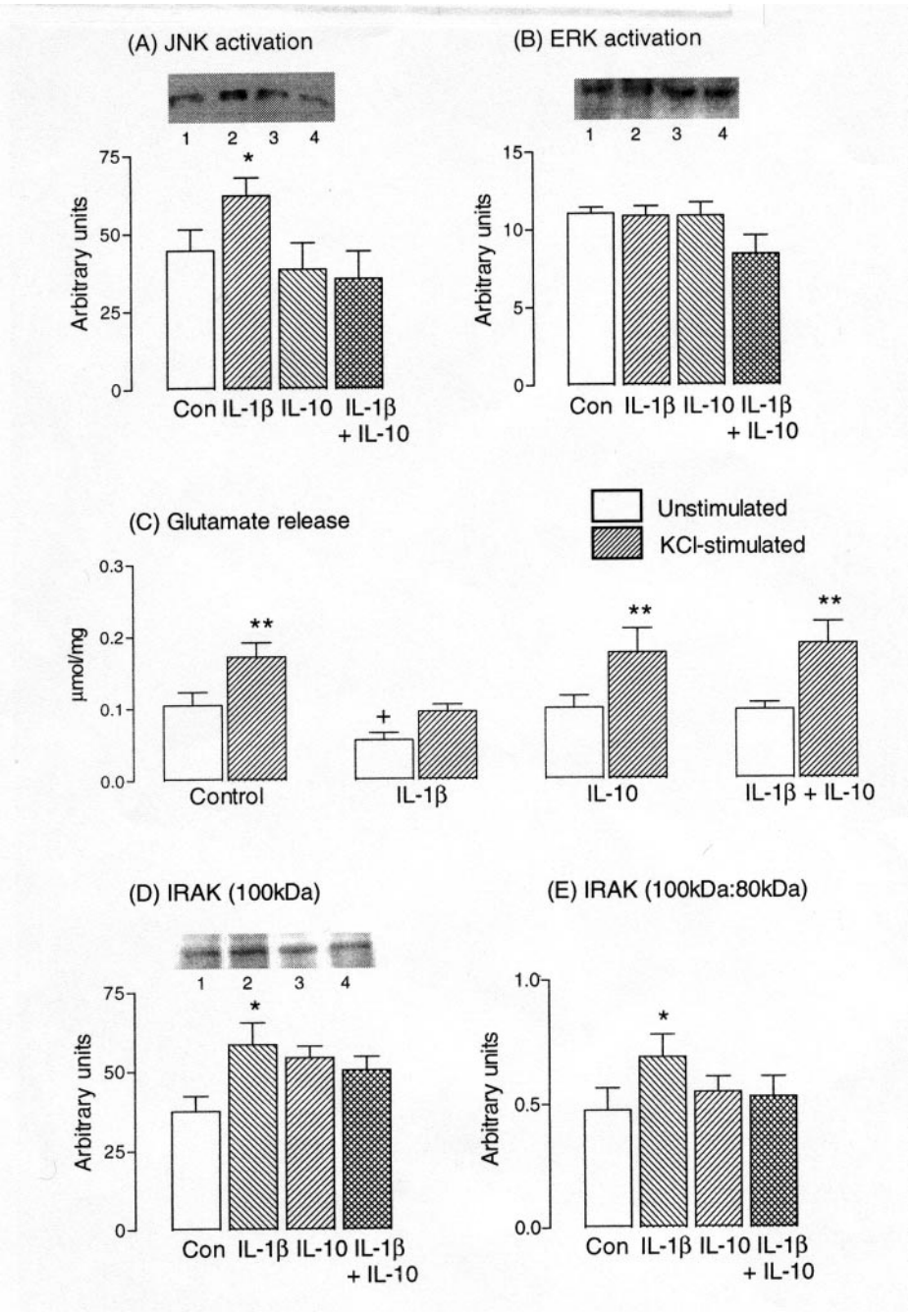

FIG. 1. IL-1β significantly increased activity of JNK (*A*, $p < 0.05$; Student's *t* test for paired means), but not ERK (*B*), in hippocampal synaptosomes, and this effect was suppressed by IL-10. The histograms represent the means (± S.E.) of 7 observations; the data were calculated by densitometric analysis and are expressed as arbitrary values. Sample immunoblots are shown in which the effect of IL-1β is shown in the presence (*lane 4*) or absence (*lane 2*) of IL-10; *lane 1* represents the control (*Con*) condition and *lane 3* the effect of IL-10 alone. *C,* KCl (40 mM) significantly increased glutamate release (**, $p < 0.01$; Student's *t* test for paired samples; $n = 6$), but this effect and unstimulated glutamate release were inhibited by preincubation of synaptosomes in IL-1β (1 ng/ml; +, $p < 0.05$ compared with untreated synaptosomes; Student's *t* test for unpaired samples). Preincubation with IL-10 (10 ng/ml) reversed the inhibitory effect of IL-1β on release, whereas release in the presence of IL-10 alone was comparable with the control. *D,* IL-1β increased expression of the 100-kDa phosphorylated form of IRAK as indicated by the sample immunoblot (compare *lanes 1* and *2*, control and IL-1β-treated, respectively). Coincubation in the presence of IL-10 (*lane 4*) reversed the IL-1β-induced effect, whereas IL-10 alone (*lane 3*) did not markedly change protein expression. Statistical analysis of the mean values obtained from densitometric analysis indicated that IL-1β significantly increased expression of IRAK ($p < 0.05$; ANOVA) but that mean values in the other 3 groups were similar. *E,* analysis of the data obtained by calculating the ratio of the 100-kDa phosphorylated form of IRAK to the 80-kDa unphosphorylated form revealed an IL-1β-induced significant increase that was reversed by coincubation in the presence of IL-10.

this effect is demonstrated in one sample immunoblot and also in the mean data obtained from seven experiments that indicate a statistically significant effect of IL-1β ($p < 0.05$; Student's *t* test for paired means). IL-10 reversed the stimulatory effect of IL-1β on JNK activity. In contrast to its effect on JNK, neither IL-1β alone nor in combination with IL-10 affected ERK phosphorylation (Fig. 1*B*).

Data from previous experiments indicated that IL-1β inhibited KCl-stimulated [^{3}H]glutamate release in hippocampus (7) and that increased JNK activity was coupled with decreased endogenous glutamate release (14, 16); therefore, we analyzed the effect of IL-1β (1 ng/ml) alone and in the presence of IL-10 (10 ng/ml) on endogenous glutamate release. Fig. 1*C* indicates that although incubation of hippocampal synaptosomes in the presence of 40 mM KCl significantly enhanced glutamate release (**, $p < 0.01$; ANOVA), this effect was blocked when IL-1β was incubated in the incubation medium. The data also indicate that unstimulated release was decreased by IL-1β (+, $p < 0.05$; ANOVA). IL-10 completely abrogated the effects of IL-1β so that both unstimulated and KCl-stimulated release were similar to values observed under control conditions.

Because JNK activation is reported to be closely coupled with IRAK phosphorylation in certain cell types (21), we addressed the question of a similar coupling in hippocampus and reported that, although IL-1β significantly increases the 100-kDa phosphorylated form of IRAK (Fig. 1*D*; $p < 0.05$; ANOVA), IL-10 inhibits this IL-1β-associated effect. When the 80-kDa unphosphorylated form of IRAK was assessed, no significant change with IL-1β, IL-10, or the combination of

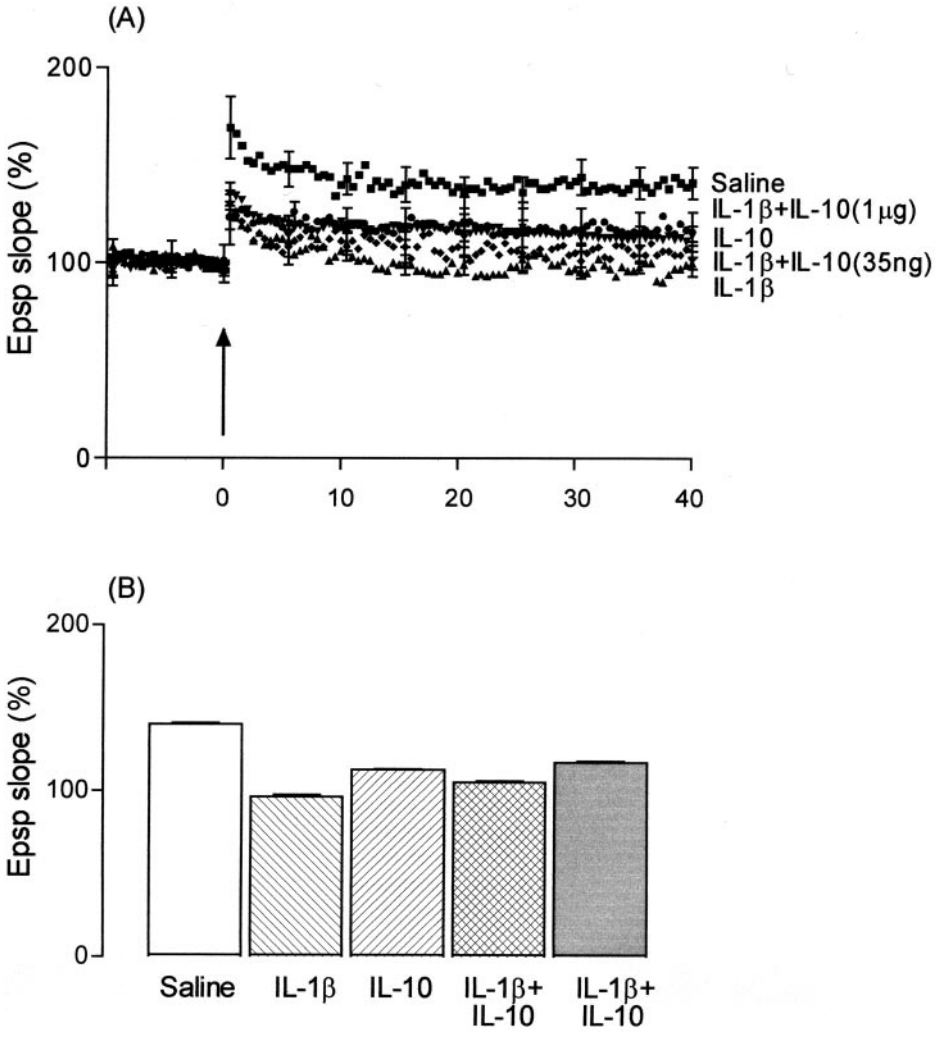

FIG. 2. Intracerebroventricular injection of IL-1β (3.5 ng/ml) inhibits both the early and late response to tetanic stimulation (*A, arrow*). The inhibitory effect of IL-1β was dose-dependently antagonized by intracerebroventricular injection of IL-10 (35 ng/ml and 1 μg/ml), whereas IL-10 also attenuated the response to tetanic stimulation. *B,* the mean percentage changes in epsp slope in the last 5 min of the experiment are compared in histogram form, and this reveals that there was a significant inhibitory effect of IL-1β ($p < 0.001$) but that this effect was significantly reversed by both 35 ng/ml ($p < 0.01$; ANOVA) and 1 μg/ml ($p < 0.001$; ANOVA) IL-10. However an inhibitory effect of IL-10 is also observed, although this effect was less marked than that of IL-1β ($p < 0.001$; ANOVA). Values are the means of 5 or 6 experiments in each group and are expressed as the percentage change in population epsp slope after tetanic stimulation (compared with the mean value immediately prior to tetanic stimulation).

both was observed (data not shown); however, analysis of the ratio of 100-kDa IRAK to 80-kDa IRAK revealed a significant increase with IL-1β which was suppressed by IL-10 (Fig. 1*E*; $p < 0.05$; ANOVA). The data are consistent with the idea that JNK activation by IL-1β and the inhibition of this effect by IL-10 is linked with, and may be a consequence of, IRAK activation.

The inhibitory effect of IL-1β on glutamate release represents one factor that might contribute to its inhibitory effect on LTP. It might be argued that, if this is the case, the effect of IL-1β on LTP may also be suppressed by IL-10. Fig. 2 indicates that, in saline-treated rats, there was an immediate increase in the population epsp slope following tetanic stimulation and that this increase persisted for the duration of the experiment. The mean percentage change (± S.E.) in the last 5 min of the experiment was 140.14 ± 2.47 (compared with the value in the 5 min prior to the tetanus). Intracerebroventricular injection of IL-1β inhibited both the early and later components of LTP; in this group, the mean percentage change in population epsp slope in the last 5 min of the experiment was 96.44 ± 1.30 (Fig. 2*B*; $p < 0.001$; ANOVA). IL-10 attenuated the effect of IL-1β in a dose-dependent manner; thus the mean percentage changes in population epsp slope (± S.E.) in the last 5 min of the experiment were 105.5 ± 0.75 and 117.3 ± 0.91, respectively, in rats treated with IL-1β and 35 ng/ml IL-10 and in rats treated with IL-1β and 1 μg/ml IL-10; these values were significantly different from the value in IL-1β-treated rats ($p < 0.001$; ANOVA).

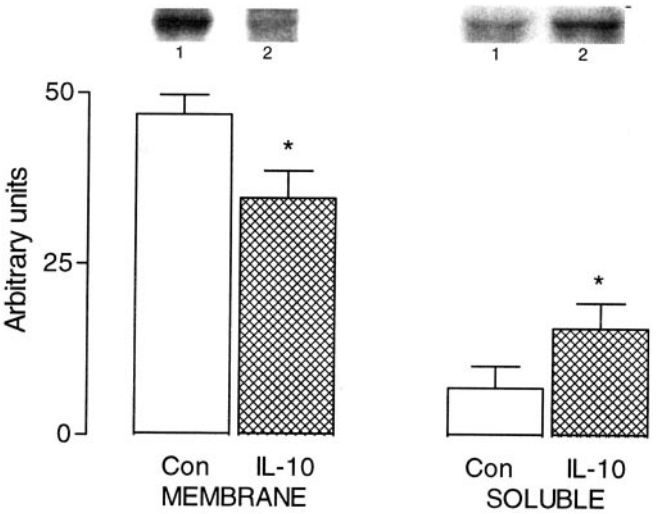

FIG. 3. Incubation of dentate gyrus in the presence of IL-10 (1 μg/ml) significantly decreased expression of membrane IL-1R1 ($p < 0.05$; Student's *t* test for paired values; compare *lane 1* (control (*Con*)) with *lane 2* (*IL-10*)) and significantly increased expression of cytosolic IL-1R1 ($p < 0.05$; Student's *t* test for paired values; compare *lane 1* (control) with *lane 2* (IL-10)).

However, the data also show that IL-10 exerted an inhibitory effect on LTP; the mean percentage change in the last 5 min of the experiment in IL-10-treated rats was 113.1 ± 0.37,

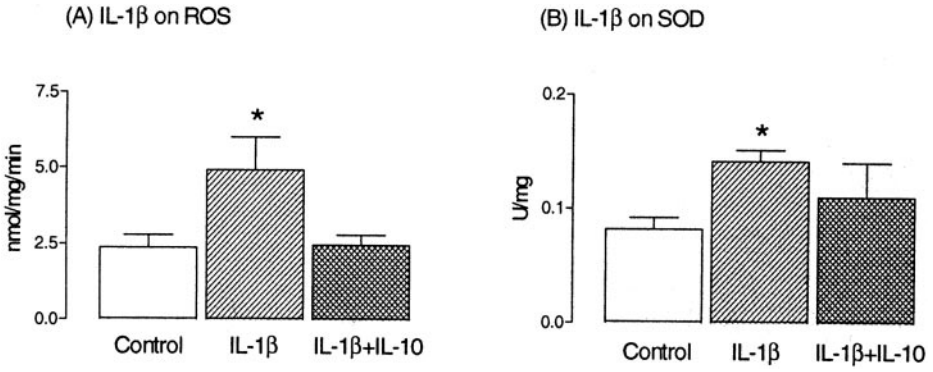

FIG. 4. IL-1β (1 ng/ml) significantly enhanced reactive oxygen species production (*A,* ROS) and superoxide dismutase (*B,* SOD) activity in hippocampus ($p < 0.05$ in both cases; Student's t test for paired samples; $n = 6$). IL-10 (10 ng/ml) blocked the effect of IL-1β on both measures.

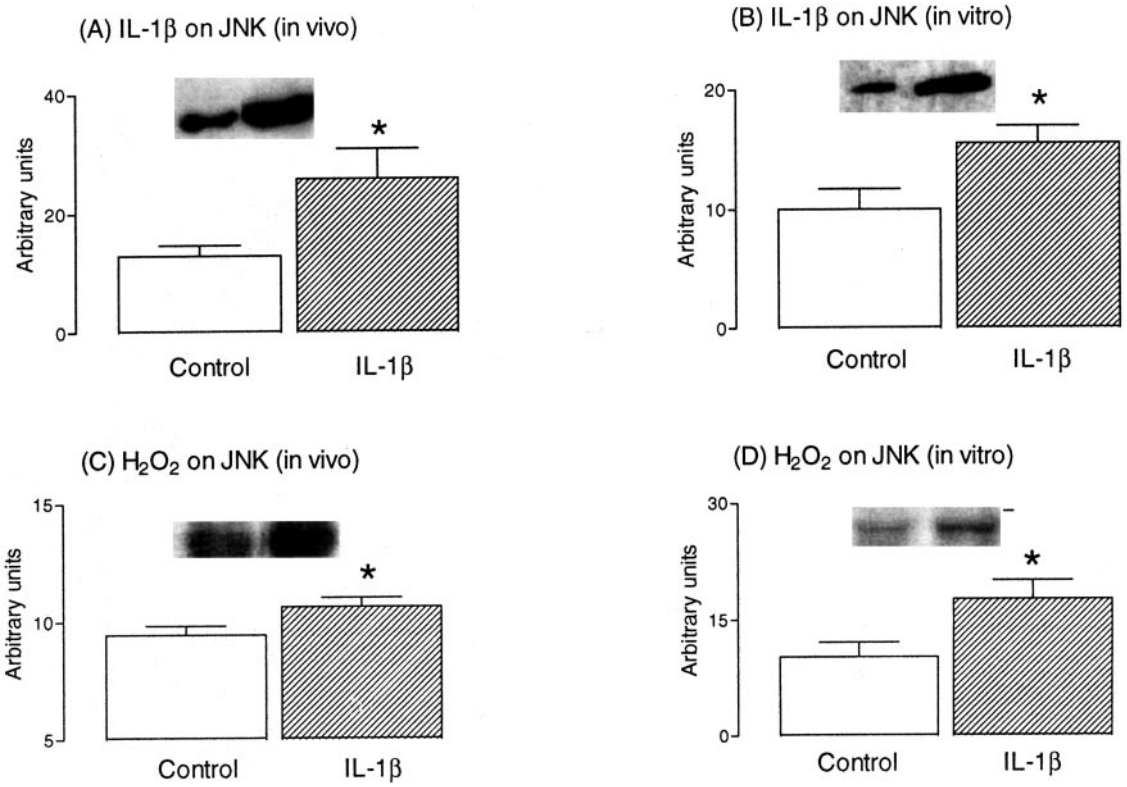

FIG. 5. Intracerebroventricular injection of IL-1β (*A,* 3.5 ng/ml) or H_2O_2 (*C,* 200 μM) significantly increased activation of JNK in hippocampal tissue ($p < 0.05$; Student's t test for independent means). This effect was mimicked *in vitro*, when IL-1β (*B,* 1 ng/ml) or H_2O_2 (*D,* 200 μM) was included in the incubation medium ($p < 0.05$; Student's t test for independent means). Mean values (arbitrary units) obtained from densitometric analysis of at least 6 replicate experiments are presented. In each sample immunoblot the stimulatory effect of either IL-1β or H_2O_2 (*right-hand lane*) is shown.

which was significantly lower than that in saline-treated controls ($p < 0.001$; ANOVA).

We considered that one mechanism by which IL-10 might act to inhibit the effect of IL-1β was by modulating expression of IL-1R1. Fig. 3 indicates that incubation of tissue in the presence of IL-10 significantly decreased expression of IL-1R1 in membrane fractions ($p < 0.05$; Student's t test for paired means) and significantly increased its expression in cytosolic fractions ($p < 0.05$; Student's t test for paired means).

Because previous data indicated that IL-1β increased reactive oxygen species production in hippocampus (12, 13), it seemed reasonable to propose that IL-10 might antagonize this effect. Fig. 4*A* indicates that IL-1β significantly increased reactive oxygen species accumulation ($p < 0.05$; Student's t test for paired values), and this was reversed by co-incubation in the presence of IL-10 (10 ng/ml). In parallel, IL-1β significantly increased superoxide dismutase activity ($p < 0.05$; Student's t test for paired values; Fig. 4*B*), and this effect was also reversed by IL-10, although it is acknowledged that the S.E. values in this case are rather large.

We report that H_2O_2 mimicked the stimulatory effect of IL-1β on JNK; thus intracerebroventricular injection of IL-1β (3.5 ng/ml; Fig. 5*A*) or H_2O_2 (200 μM; Fig. 5*C*) increased JNK activity as shown by the sample immunoblots; analysis of the mean values obtained from densitometric analysis indicated a significant stimulatory effect of both agents ($p < 0.05$; Student's t test for paired values). These effects of IL-1β and H_2O_2 were mimicked *in vitro*; thus incubation of hippocampal tissue in the presence of IL-1β (Fig. 5*B*) or H_2O_2 (Fig. 5*D*) increased JNK as indicated by the sample immunoblots; densitometric analysis revealed that these effects were statistically significant ($p < 0.05$; Student's t test for paired values).

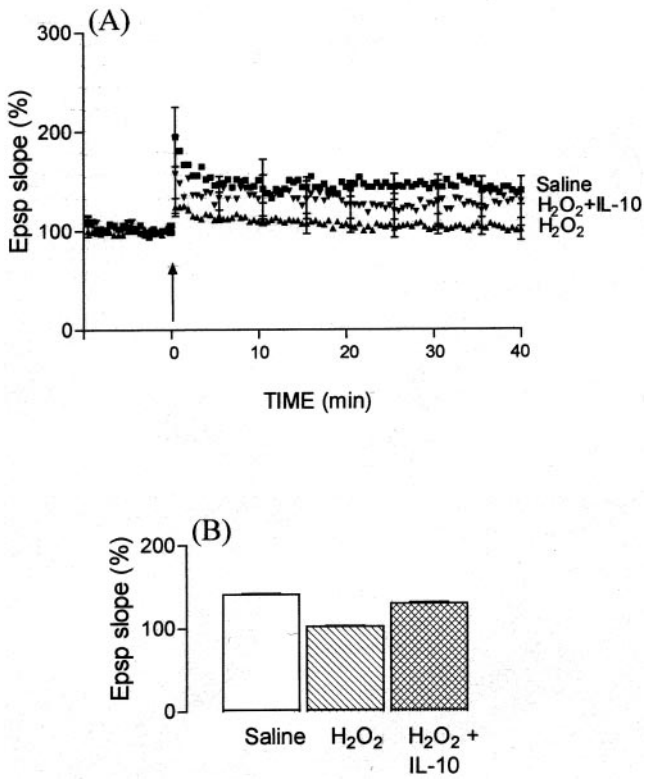

FIG. 6. **Intracerebroventricular injection of H_2O_2 (200 μM) inhibits both the early and late response to tetanic stimulation (*arrow*).** *A*, the inhibitory effect of H_2O_2 was antagonized by intracerebroventricular injection of IL-10 (1 μg/ml). The values presented are means of at least five determinations. *B,* the mean percentage changes in epsp slope in the last 5 min of the experiment are compared in histogram form, and this reveals that there was a significant inhibitory effect of H_2O_2 ($p < 0.001$; ANOVA) but that this effect was significantly reversed by both 1 μg/ml IL-10 ($p < 0.001$; ANOVA; comparison of the effect of H_2O_2 with and without IL-10).

If IL-1β mediates its effects by increasing reactive oxygen species production, and if IL-10 inhibits the effect of IL-1β by antagonizing this, then the inhibitory effect of IL-1β on LTP should be mimicked by H_2O_2, which generates reactive oxygen species, and IL-10 should suppress this effect of H_2O_2. Fig. 6*A* indicates that LTP was induced and sustained in saline-treated rats but blocked in rats that received an intracerebroventricular injection of H_2O_2; the mean percentage changes in population epsp slope (± S.E.) in the last 5 min of the experiment (compared with the value in the 5 min prior to the tetanus) were 141.2 ± 0.81 and 102.4 ± 0.83 in saline-treated and H_2O_2-treated rats, respectively (Fig. 6*B*). Intracerebroventricular injection of IL-10 (1 μg/ml) reversed the inhibitory effect of H_2O_2, but values were not completely restored to control values; thus the mean percentage change in population epsp slope in the last 5 min of the experiment was 129.9 ± 1.58 (Fig. 6*B*).

Both IL-1β and H_2O_2 induced parallel changes in JNK activation and glutamate release, but confirmation of a causal relationship between the two measures requires assessment of the effect of a JNK inhibitor on IL-1β-induced inhibition of glutamate release. Although not specific, VIP has been shown to inhibit JNK in some cells, and here we assessed the possibility that it might inhibit IL-1β- and H_2O_2-induced JNK activation in hippocampus. Fig. 7, *A* and *C,* shows sample immunoblot in which the stimulatory effect of IL-1β (Fig. 7*A*) and H_2O_2 (Fig. 7*C*) on JNK phosphorylation are clearly shown (compare *lanes 1* and *2*; control and IL-1β- or H_2O_2-treated, respectively); these sample immunoblots also show that VIP inhibited the IL-1β- and H_2O_2-induced effects (*lane 4*), whereas VIP alone exerted no marked effect (*lane 3*). Densitometric analysis allowed comparison of the data obtained from 5 or 6 replicate experiments; statistical analysis of these data revealed significant increases in JNK activity induced by IL-1β and H_2O_2 ($p < 0.05$; ANOVA in each case) and VIP-associated reversal of these stimulatory effects. In an effort to establish a causal relationship between IL-1β- and H_2O_2-induced inhibition of glutamate release and JNK activation, we investigated the effect of VIP on IL-1β- and H_2O_2-induced inhibition of glutamate release. Fig. 7, *B* and *D,* shows that the inhibitory effects of both IL-1β and H_2O_2 on KCl-stimulated glutamate release were blocked by VIP. Thus incubation of synaptosomes in the presence of KCl significantly enhanced glutamate release in control conditions and when tissue was incubated in the presence of IL-1β and VIP or and H_2O_2 and VIP ($p < 0.05$; ANOVA in all cases). However, the effect of KCl was inhibited by IL-1β or and H_2O_2 and to a lesser extent by incubation in the presence of VIP alone.

DISCUSSION

We set out to investigate whether the anti-inflammatory cytokine, IL-10, might reverse the inhibitory effects of the proinflammatory cytokine, IL-1β, on synaptic function in the hippocampus. The data indicate that the IL-1β-induced inhibition of LTP in perforant path-granule cell synapses was abrogated by IL-10, and the evidence suggests that this is likely to be a consequence of the ability of IL-10 to overcome the coupled stimulatory effects of IL-1β on reactive oxygen species production and JNK activity. Our evidence is consistent with the idea that these changes might be consequent upon the IL-10-induced decrease in expression of membrane IL-1R1.

IL-1β significantly attenuated unstimulated and KCl-stimulated release of endogenous glutamate in hippocampal synaptosomes. This is consistent with a previous report (7) from this laboratory in which we observed an inhibitory effect of IL-1β on release of radiolabeled glutamate. IL-10 completely reversed the inhibitory effect of IL-1β on both unstimulated and KCl-stimulated glutamate release. In parallel, the stimulatory effect of IL-1β on JNK activation in hippocampal tissue was blocked by co-incubation in the presence of IL-10. We have reported previously (14, 16) that IL-1β stimulated JNK activity in hippocampal tissue, supporting the findings of others (19) in a variety of different cell types. To our knowledge, this is the first report indicating that IL-10 suppresses the activation of JNK by IL-1β in brain tissue. The findings (*a*) that JNK activity and glutamate release are negatively correlated (14, 16), and (*b*) that IL-10 blocks both the stimulatory effect of IL-1β on JNK and the inhibitory effect on release suggest that activation of JNK may be directly responsible for impaired glutamate release. We have attempted to address this question more thoroughly by investigating the effect of VIP, which has been shown to inhibit the effect of JNK in several cell types including macrophages (39), on glutamate release. We established that, in parallel with its ability to inhibit IL-1β-induced JNK activation in hippocampus, as in other cells (39), VIP suppressed the inhibitory effect of IL-1β on glutamate release. These findings provide further evidence that JNK activation negatively impacts on glutamate release and that this effect may be the root of the inhibitory effect of IL-1β on release.

The findings of previous studies have suggested that IRAK phosphorylation, which occurs downstream of IL-1R1 activation, is closely linked with JNK activation (21), and in an effort

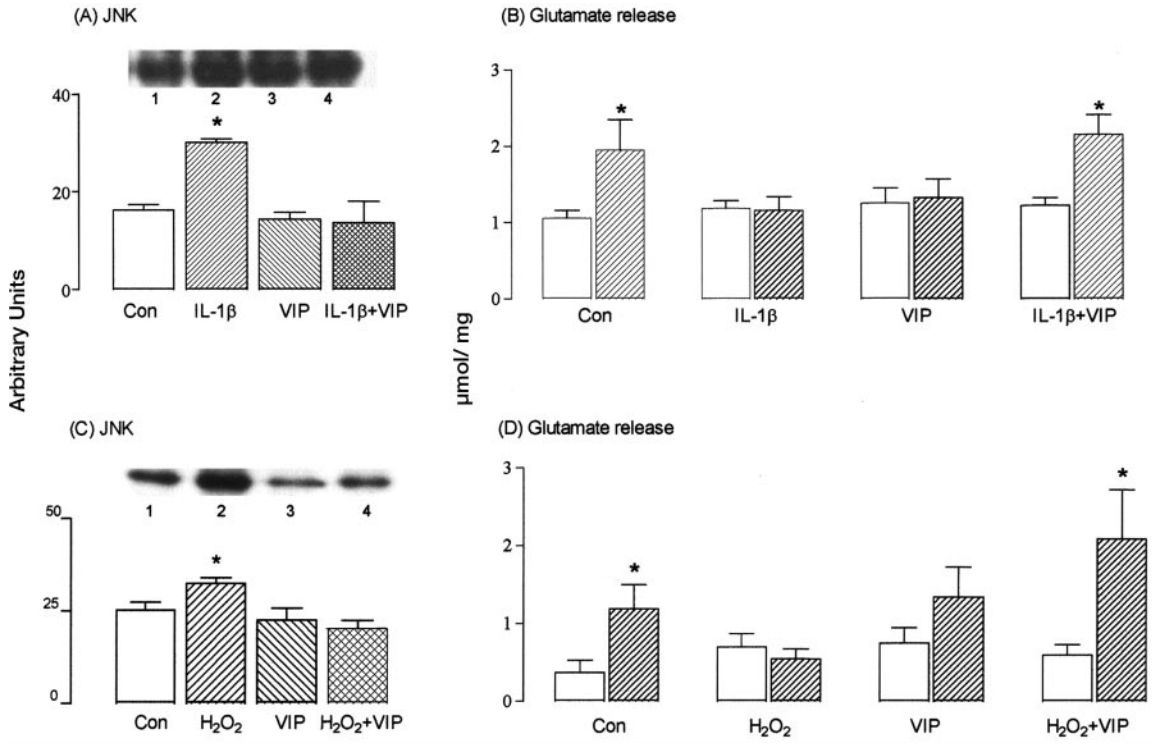

FIG. 7. **The IL-1β-induced and H_2O_2-induced (*A* and *C*) increases in JNK activation and decreases in KCl-stimulated glutamate release (*B* and *D*) are abrogated by VIP.** *A,* IL-1β (1 ng/ml) and H_2O_2 (200 μM) significantly increased JNK activation ($p < 0.05$; ANOVA), and this effect was blocked by vasoactive intestinal peptide (1 μM), which alone exerted no effect. The stimulatory effects on JNK activation are shown in the two sample immunoblots (compare *lane 2* with *lane 1* in *A* and *C*); these effects contrast with the lack of change following incubation in the presence of VIP alone (*lane 3*) or VIP combined with IL-1β or H_2O_2 (*lane 4*). *B* and *D*, addition of KCl (40 mM) to the incubating medium significantly enhanced glutamate release in synaptosomes prepared from dentate gyrus ($p < 0.05$; ANOVA), and this effect was inhibited by IL-1β (1 ng/ml) and H_2O_2 (200 μM). VIP (1 μM) suppressed the IL-1β-induced and H_2O_2-induced inhibition of glutamate release so that the KCl-associated stimulatory effect was restored, but VIP alone also exerted an inhibitory effect on KCl-stimulated release.

to establish this coupling in hippocampal tissue, we investigated the change in expression of the phosphorylated 100-kDa form of IRAK in hippocampal tissue that had been incubated in IL-1β with/without IL-10. The data indicated that, in parallel with the IL-1β-induced enhancement of JNK phosphorylation, IL-1β also increased expression of the phosphorylated form of IRAK and concomitantly increased the ratio of phosphorylated to unphosphorylated IRAK. These observations suggested that the primary effect of IL-10 was upstream of JNK activation, at least at the level of IRAK activation. Indeed the present finding that IL-10 decreased expression of membrane-associated IL-1R1 suggests that the primary effect of IL-10 may be to induce shedding of the IL-1R1 in a manner that may be analogous to shedding of the decoy IL-1RII (40). It seems reasonable to propose that a reduction in membrane IL-1R1 will lead to down-regulation of downstream signaling events and may explain the ability of IL-10 to block IL-1β-induced activation of JNK (14, 16).

Results from previous studies (14, 16) have indicated that LTP in perforant path-granule cell synapses is accompanied by an increase in glutamate release and that LTP is impaired when glutamate release is inhibited and when JNK is activated. Thus we have reported that the compromised LTP in aged rats (16), rats treated intracerebroventricularly with IL-1β (14), or rats treated intraperitoneally with lipopolysaccharide (41) is accompanied by increased JNK activation and decreased release. We argued that if increased JNK activation and decreased glutamate release are responsible for IL-1β-induced inhibition of LTP, then IL-10, which reverses these effects of IL-1β *in vitro*, should block the inhibitory effect of IL-1β on LTP. The data presented here confirm our previous observations that IL-1β inhibits LTP (12–14, 16) and demonstrate that IL-10 acts in a dose-dependent manner to abrogate this effect of IL-1β. We propose that the inhibitory effect of IL-10 on IL-1β-induced changes *in vitro* suggests that IL-10 may mediate its effect by blocking the stimulatory effect of IL-1β on JNK and its inhibitory effect on glutamate release. Three previous studies are relevant to this observation. First, it has been reported that when anergy was induced in a T cell line, IL-10 production was increased, whereas JNK activity was blocked (42), suggesting a negative correlation between IL-10 and JNK activity, which is consistent with the present findings. Second, it has been shown that, in monocytes, IL-10 inhibited LPS-induced increase in activation of another mitogen-activated protein kinase, p38 (43). Third, IL-10 has been shown to inhibit the effect of TNFα in monocyte-derived dendritic cells, and this antagonism has been attributed to the ability of IL-10 to block the effect of TNFα on mitogen-activated protein kinases, including JNK (44).

In addition to the finding that IL-10 abrogates the inhibitory effect of IL-1β on JNK activation, the results of this study suggest that IL-10 may exert antioxidant effects. We observed that IL-1β increased reactive oxygen species production in the hippocampus and that this effect was suppressed by IL-10. This effect of IL-10 is consistent with earlier findings indicating a negative correlation between IL-10 and reactive oxygen species production in leukocytes (45). Indeed it has been proposed (46) that the protective role of IL-10 following an acute inflammatory stimulus might be associated with its ability to limit superoxide accumulation in endothelial cells. The present data suggest that this role of IL-10 might be a consequence of its ability to overcome the

IL-1β-induced increase in activity of superoxide dismutase. The stimulatory effect of IL-1β on superoxide dismutase in hippocampus concurs with earlier data that demonstrated that the cytokine up-regulated Mn-superoxide dismutase gene expression in cultured rat hepatocytes (47) and increased the activities of both Mn- and Cu/Zn-superoxide dismutases in rat pancreatic islets (48).

It is significant that H_2O_2, which generates reactive oxygen species production (16, 49), mimics the effect of IL-1β in hippocampus in at least two respects; first, it activates JNK activity both *in vivo* and *in vitro,* and second, it also inhibits LTP. It has been known for several years that JNK activity is up-regulated in response to stress, including oxidative stress (17, 22, 50), and therefore the finding that intracerebroventricular injection of H_2O_2 activated JNK in hippocampus was not surprising; indeed the stimulatory effect of H_2O_2 on JNK activation *in vitro* confirms our earlier observations (14, 16).

Intracerebroventricular injection of H_2O_2 inhibited both the early and later responses to tetanic stimulation of the perforant path. The effect of H_2O_2 administration on LTP *in vivo* or its effect in dentate gyrus has not been reported previously, although it has been shown to inhibit LTP in guinea pig CA1 *in vitro* (51), and we have previously coupled an increase in reactive oxygen species accumulation with a compromise in LTP in dentate gyrus of aged rats and following intracerebroventricular injection of IL-1β (12, 13). The present data indicate that IL-10 blocked the inhibitory effect of H_2O_2 on LTP. Thus IL-1β induced reactive oxygen species production and H_2O_2 mimicked the effect of IL-1β in inhibiting LTP and activating JNK, and these effects were reversed by IL-10. On the basis of these observations, it seems reasonable to propose that IL-1β may exert its effects by increasing reactive oxygen species production leading to JNK activation. Confirmation of a pivotal role for JNK in inhibition of glutamate release and LTP must await the availability of a JNK inhibitor; however, our observation that VIP suppressed the inhibitory effects of IL-1β and H_2O_2 provides preliminary evidence of a causal link between activation of JNK and inhibition of glutamate release.

Although the data presented in this study represent a significant advance in our understanding of the mechanism of action of IL-10, it must be acknowledged that a few previous studies have reported that IL-10 opposes the effect of IL-1. For example, IL-10 has been shown to reduce tissue damage following experimentally induced traumatic brain injury, and because this was associated with lower IL-1 expression, it was concluded that this was due to an inhibition of IL-1 expression by IL-10 (32). Similarly, injection of IL-10 reduced LPS-induced fever (29) and the behavioral effects (52) induced by LPS. Because these effects of LPS are also attributed to increased production and/or release of IL-1, these observations have been interpreted as an indication of the ability of IL-10 to counteract the effect of IL-1. It remains to be established whether, in the latter two studies, IL-10 inhibited production of IL-1 as it does in macrophages (24) or whether it antagonizes the effect of IL-1. In this context, it is significant that IL-10 (at the same concentration used in the present study) has been shown to inhibit the IL-1β-induced increase in IL-6 production in astrocytes (53) and RANTES (regulated on activation normal T cell expressed and secreted) mRNA expression in microglia (54).

Our data demonstrate that the inhibitory effects of IL-1β on hippocampal synaptic function can be abrogated by the anti-inflammatory cytokine, IL-10. We propose that this action is dependent on its ability to overcome the pro-oxidant effects of IL-1β that lead to activation of JNK and the consequent inhibition of glutamate release and LTP.

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