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ACCELERATED COMMUNICATION

Interleukin-1 Augments γ-Aminobutyric Acid$_A$ Receptor Function in Brain

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SUMMARY

Interleukin-1 (IL-1), a cytokine involved in the acute phase reaction to injury and infection, has multiple effects in the central nervous system, including induction of fever and sleep and the release of several neuropeptides. We evaluated effects of IL-1β on inhibitory postsynaptic function at the γ-aminobutyric acid$_A$ (GABA$_A$) receptor. IL-1 (100 pg/ml to 10 ng/ml) augmented GABA$_A$ receptor function in cortical synaptic preparations. This effect of IL-1 was largely prevented by incubation with a specific IL-1 receptor antagonist. The related cytokines interleukin-6 and tumor necrosis factor did not augment GABA-dependent chloride transport. Similar enhancement of GABA$_A$ receptor function was observed in tissue prepared from mice previously injected intraperitoneally with IL-1 (1 μg). Electrophysiological studies in cultured primary cortical neurons demonstrated that IL-1 enhanced the GABA-mediated increase in chloride permeability, whereas IL-1 alone produced no alterations in resting conductance. Behavioral studies indicated that IL-1 is similarly active *in vivo*; mice treated with IL-1 showed a decrease in open-field activity and an increase in the threshold for pentylenetetrazol-induced seizures. The interaction of IL-1 with GABA$_A$ receptors might account for the somnogenic and motor-depressant effects of this cytokine.

IL-1 is a polypeptide cytokine that mediates several components of the acute phase response of host defense (1). IL-1 affects central nervous system function, including the induction of sleep and fever and the secretion of adrenocorticotropic hormone from the hypothalamus (2–4). IL-1 is present in brain in several species and has been localized to neurons histochemically (5). This endogenous IL-1 appears to be synthesized by astrocytes and microglia rather than by neurons (6, 7). In addition, IL-1 receptors are present in brain, although in low abundance (8). In view of the inhibitory nature of IL-1 effects such as somnogenesis, we investigated the effects of IL-1 on the postsynaptic actions of GABA, the major inhibitory neurotransmitter in brain. Our results indicate that IL-1β, but not the related cytokines IL-6 and TNF, augments GABA$_A$ receptor function in neurochemical and electrophysiological paradigms. Effects of IL-1 occur both *in vitro* and *in vivo*.

Materials and Methods

Male CD1 mice (6–8 weeks of age) were purchased from Charles River Laboratories (Wilmington, MA). Recombinant human IL-1β was provided by Dr. Aldo Tagliabue (Sclavo SA, Siena, Italy), recombinant human TNF was provided by Genentech (South San Francisco, CA), and IL-6 was provided by Dr. Steven Clark (Genetics Institute, Cambridge, MA). IL-1 receptor antagonist was provided by Dr. Daniel Tracey (Upjohn, Kalamazoo, MI). This protein was generated by expression of the cDNA in *Escherichia coli* (9). Purity was greater than 95%.

GABA-dependent chloride uptake. GABA-dependent chloride uptake was performed as previously described (10). Briefly, cortical synaptoneurosomes were prepared and resuspended in assay buffer (145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM HEPES, pH 7.4). Synaptoneurosomes were incubated with IL-1 (10 pg/ml to 10 ng/ml) or vehicle for 10 min at 30° before addition of muscimol (1–50 μM), or IL-1 was added simultaneously with muscimol. Results were similar with the two methods. To 100 μl of membranes were added 100 μl of a solution containing muscimol (1–100 μM) and $^{36}Cl^-$ (0.2 μCi/ml of assay buffer). After 6 sec the incubation was terminated by addition of 0.5 ml of cold assay buffer, containing 6 μM picrotoxin, and filtration on Whatman GF/C filters, using a Brandel M24 apparatus. Filters were washed twice with cold buffer and quantitated by scintillation counting.

Open-field activity. Activity was determined in mice over a 5-min interval between 9 and 11 a.m., using an Omnitech Digiscan apparatus (10).

Pentylenetetrazol-induced seizures. Mice were pretreated with IL-1 (1 μg intraperitoneally) or vehicle, and pentylenetetrazol (10 mg/

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ABBREVIATIONS: IL-1, interleukin-1; GABA, γ-aminobutyric acid; TNF, tumor necrosis factor; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

ml) was infused intravenously (0.6 ml/min) after 30 min (11). Infusion was stopped at the onset of a tonic-clonic seizure.

Electrophysiological studies. Neurons were dissociated from 8-day chick embryos and cultured as previously described (12). Electrophysiological studies were carried out on cells after 4–5 days in culture, using standard tight-seal whole-cell recording techniques (13). The neurons were clamped at a holding potential of −60 mV while they were tested with GABA. Drugs were applied by pressure ejection from blunt-tipped puffer pipettes (3 μm o.d.) positioned approximately 20 μm from the cell under study. Removal of the drugs was achieved by diffusion in the bulk solution (approximately 10-fold decrease in concentration/min).

Results and Discussion

Initial experiments evaluated the effects of IL-1β (henceforth IL-1) on GABA-dependent chloride uptake in cortical synaptoneurosomes *in vitro*, using the GABA analog muscimol. Incubation of tissue with IL-1 (10 ng/ml) for 10 min before uptake produced a 30–40% increase in uptake at the highest doses of muscimol evaluated (Fig. 1A). The effects of IL-1 were dose dependent; no alterations were observed at the lowest dose evaluated, 10 pg/ml, and a small nonsignificant effect occurred at 100 pg/ml. Enhancement of uptake was similar with 1 and 10 ng/ml. Similar results were obtained when IL-1 was added to the tissue simultaneously with muscimol at the onset of the uptake interval. In the absence of exogenous muscimol, various concentrations of IL-1 had no effect on chloride uptake (GABA-independent chloride uptake). The increase in maximal uptake observed with IL-1 occurred without a significant alteration in the EC_{50} for muscimol.

To assess the specificity of IL-1 in augmenting GABA-dependent chloride uptake, the effects of the biologically related cytokines IL-6 and TNF were evaluated. Chloride uptake was unchanged from controls with either IL-6 or TNF (10 ng/ml) (Fig. 1B). The efficacy of IL-1 in augmenting GABA-dependent uptake might be due to indirect effects of IL-1 at its own receptor or to direct effects of IL-1 on the $GABA_A$ receptor. The availability of a specific IL-1 receptor antagonist permits evaluation of IL-1 receptor effects (9). Pretreatment of tissue with this antagonist (1 μg/ml) largely prevented the effects of IL-1 (10 ng/ml) on GABA-dependent chloride uptake (Fig. 1C). In contrast, IL-1 at concentrations up to 1 μg/ml had no effect on binding at the benzodiazepine, GABA, or *t*-butylbicyclophosphorothionate sites on the $GABA_A$ receptor complex (data not shown) (10).

These results indicate that IL-1, acting through its own receptor sites, specifically augments GABA-dependent chloride uptake in cortical synaptoneurosomes *in vitro*. IL-1 administered peripherally exerts a number of effects on the central

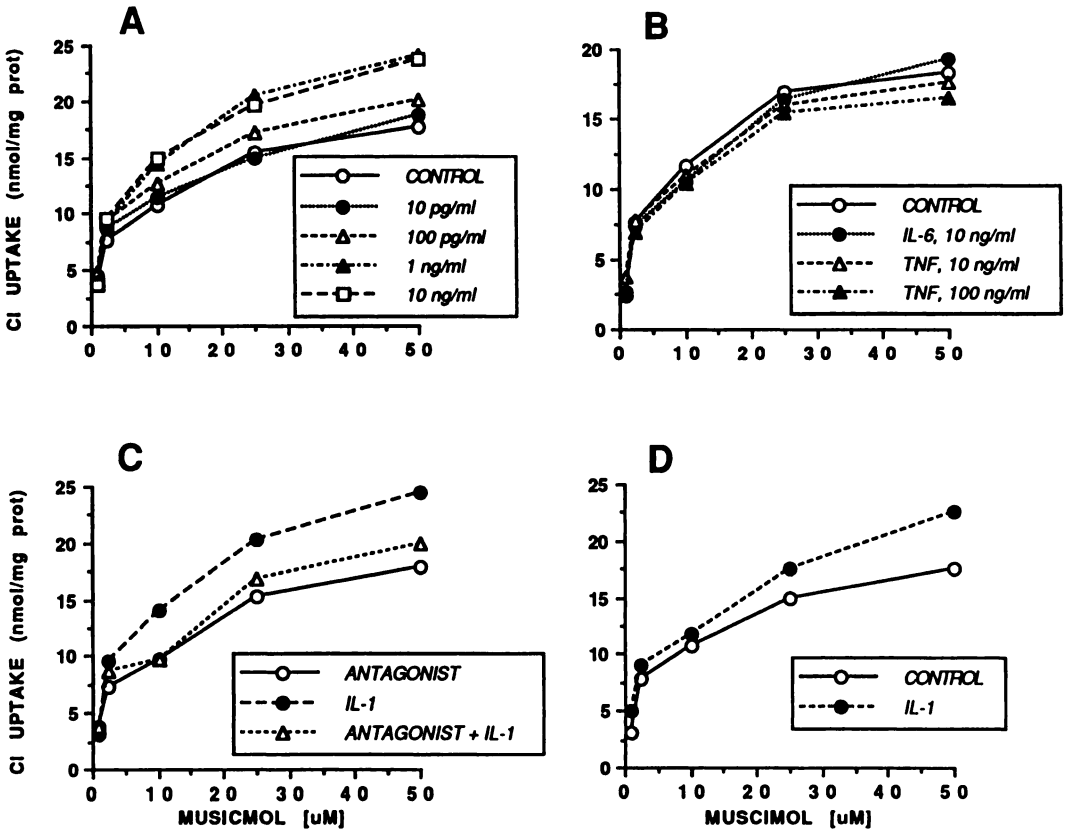

Fig. 1. Effects of cytokines on GABA-dependent chloride uptake *in vitro*. A, IL-1. Synaptoneurosomes were incubated with IL-1 for 10 min before addition of muscimol. Results are means of five to eight experiments for each concentration of IL-1. Uptake at 25 and 50 μM muscimol is greater with IL-1 (1 and 10 ng/ml), compared with controls ($p < 0.05$). The EC_{50} for muscimol is not significantly changed by IL-1. B, IL-6 and TNF. Synaptoneurosomes were incubated with IL-6 or TNF (10 ng/ml) for 10 min before addition of muscimol. Results are means of five or six experiments for each cytokine. There are no significant differences. C, IL-1 receptor antagonist. Tissue was incubated with the antagonist (1 μg/ml) or vehicle for 5 min before IL-1 incubation as above. Results are means of five experiments. There are no significant differences. D, *In vivo*. Mice were treated with IL-1 (1 μg intraperitoneally) or vehicle. After 30 min, mice were sacrificed, synaptoneurosomes were prepared, and GABA-dependent chloride uptake was determined. Results are means of six experiments. Uptake at 25 and 50 μM GABA is significantly greater than controls ($p < 0.05$).

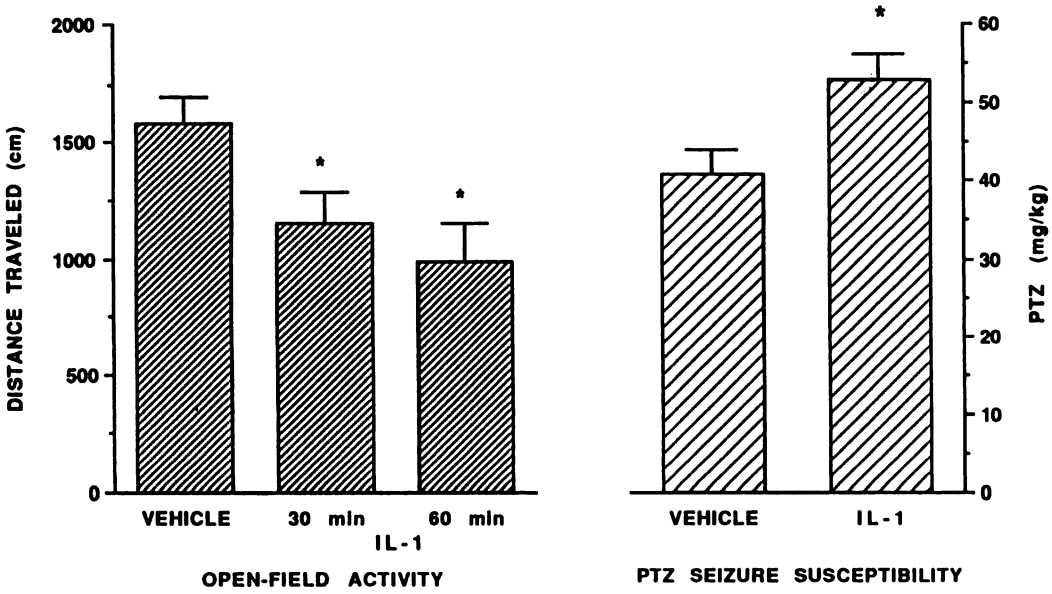

Fig. 2. Effects of IL-1 *in vivo*. A, Open-field activity. Mice were treated with IL-1 (500 ng intraperitoneally) or vehicle. Results are means ± standard errors (n = 8 or 9). Both IL-1 groups are significantly decreased, compared with controls ($p < 0.05$). B, Induced seizures. Mice were pretreated with IL-1 (1 μg intraperitoneally) or vehicle. IL-1-treated mice required more pentylenetetrazol (*PTZ*) to induce seizures, compared with control mice ($p < 0.05$).

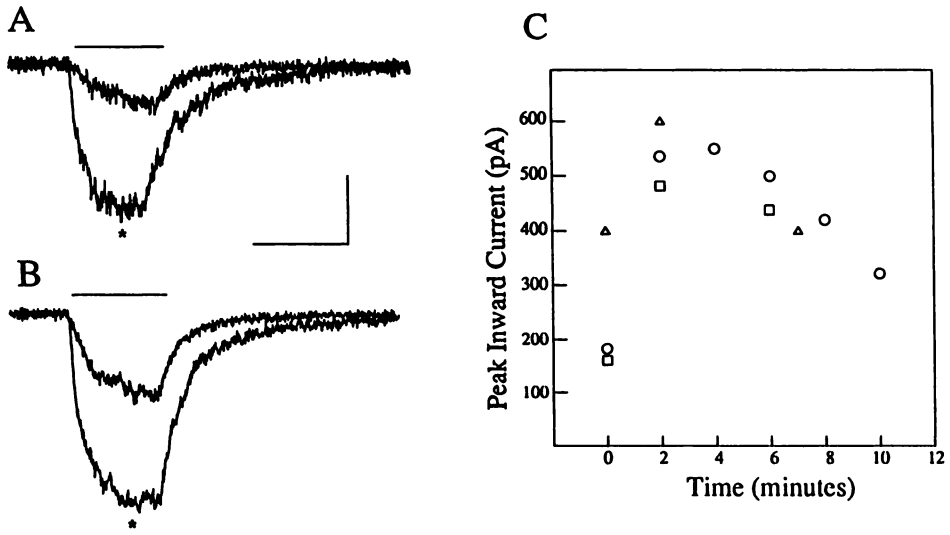

Fig. 3. IL-1-induced potentiation of GABA-mediated inward currents in cultured chick cortical neurons. A and B, Two superimposed current traces showing the inward current evoked by bath application of 10 μM GABA before and after (*) exposure of the neuron to IL-1 (10 ng/ml). The time of GABA application is shown by a *horizontal bar* above the current records. A, IL-1 and GABA were applied simultaneously. B, IL-1 was applied approximately 30 sec before GABA application. *Calibration bars*, 300 pA, 2 sec. C, Time course of the IL-1-mediated potentiation. Cortical neurons were exposed every 2 min to a 2-sec application of 10 μM GABA, and peak current was measured. At time 0, GABA alone was applied. Two minutes after this application of GABA, a 2-sec application of IL-1 was followed immediately by a GABA stimulus. Subsequent applications included GABA alone. The GABA-evoked currents remained potentiated for several minutes after the IL-1 was removed. Different symbols represent data from different neurons.

nervous system, although it is uncertain whether IL-1 crosses the blood-brain barrier or acts primarily at the circumventricular organs (14–15). To confirm these effects *in vivo* in mice and to assess whether such effects might involve the GABAergic system, IL-1 was administered peripherally to mice, and open-field activity and effects on the pentylenetetrazol-induced seizure threshhold were examined. Administration of IL-1 to mice (500 ng intraperitoneally) led to a substantial decrease in open-field activity after 30 min (Fig. 2A); a small additional decrease occurred after 60 min. IL-1 was also effective in the induced-seizure paradigm. At 30 min after IL-1 administration (1 μg intraperitoneally), mice required approximately 30% more pentylenetetrazol for induction of a tonic-clonic seizure, compared with control animals (Fig. 2B).

In view of the effects of IL-1 *in vivo* in mice, GABA-dependent chloride uptake was determined in cortical synaptoneurosomes prepared from mice pretreated with IL-1 (1 μg intraperitoneally). At 30 min after administration, chloride uptake was enhanced in IL-1-treated compared with vehicle-treated mice (Fig. 1D). Thus, effects of IL-1 on GABA-related chloride uptake occur both *in vitro* and *in vivo*.

To corroborate neurochemical findings with electrophysiological data, effects of IL-1 were studied using tight-seal whole-cell voltage-clamp techniques (13) in primary cultures of chick cortical neurons (Fig. 3). Application of GABA to the bath evoked an increase in inward holding current, which results from the opening of chloride-selective channels (17). GABA at 10 μM produced an average current increase of 363 ± 82 pA (mean ± standard error, n = 9). IL-1 potentiated the effect of GABA when it was applied either simultaneously with, or before, GABA (Fig. 3, A and B). Not all cortical neurons were sensitive to IL-1; GABA-mediated currents were potentiated in seven of nine cells tested, with effects ranging from 1.4- to 4.4-fold increases (2.4 ± 0.5, mean ± standard error). The action of IL-1 was relatively long lasting. Following a brief application of IL-1, its potentiating effects on GABA-evoked currents could be observed for approximately 10 min (Fig. 3C).

These data indicate that IL-1 enhances postsynaptic GABA

effects in the central nervous system. In both tissue preparations and cultured neurons, IL-1 augmented the effects of GABA in promoting chloride transport. The effects of IL-1 occurred both *in vitro* and *in vivo*; tissue prepared from IL-1-treated mice had similar enhancement of chloride uptake as did tissue to which IL-1 was added *in vitro*. Results of voltage-clamp experiments also indicate effects of IL-1 *in vivo*. IL-1 effects may be physiological, as supported by the decrease in open-field activity after IL-1, although this behavior is affected by several neurotransmitter systems (10). More specific evidence for the involvement of the $GABA_A$ receptor system *in vivo* comes from induced-seizure studies, which employ the specific $GABA_A$ receptor chloride channel antagonist pentylenetetrazol. It should be noted that *in vivo* effects of IL-1 may not reflect the same mechanism as effects observed *in vitro*, because IL-1 has not been demonstrated to cross the blood-brain barrier (14, 15). Effects of IL-1 *in vivo* might be due to a fragment of the molecule or to signal transduction by another system.

Both IL-1 and IL-1 receptors are present in brain (5, 8), and IL-1 has been localized to neurons histochemically (5). The endogenous brain IL-1 appears to be synthesized by astrocytes and microglia rather than neurons (6, 7). Although the mechanism for IL-1 effects on GABAergic transmission remains uncertain, our results support a specific interaction with the IL-1 receptor. This finding is based on the antagonism of IL-1 effects by the receptor antagonist, as well as the lack of effect of IL-6 and TNF. The inactivity of IL-6 in this study is consistent with the lack of somnogenic effects of this cytokine (18). However, TNF was also inactive but is somnogenic, indicating that cytokines may exert inhibitory effects through other systems. In addition, the lack of change in GABA-dependent chloride uptake in preparations exposed to the IL-1 receptor antagonist alone may indicate that endogenous IL-1 has little effect on the $GABA_A$ receptor in the basal state.

Effects of IL-1 on GABAergic function suggest that, during infection, sepsis, or injury, IL-1 might enhance host adaptation by promoting inhibition in the central nervous system. This effect of IL-1 could be protective against associated events with potential excitatory effects, such as fever and electrolyte alterations.

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