Brain type I but not type II IL-1 receptors mediate the effects of IL-1β on behavior in mice

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Cremona, Sandrine, Emmanuelle Goujon, Keith W. Kelley, Robert Dantzer, and Patricia Parnet. Brain type I but not type II IL-1 receptors mediate the effects of IL-1β on behavior in mice. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R735–R740, 1998.—In the immune system, interleukin (IL)-1β effects are mediated by the type I IL-1 receptors (IL-1RI), whereas the type II IL-1 receptors (IL-1RII) act as inhibitory receptors. IL-1RI and IL-1RII are also present in the brain. To study their functionality in the brain, mice were centrally treated with neutralizing monoclonal antibody (MAb) directed against IL-1RI (35F5, 1 µg) or against IL-1RII (4E2, 2 µg) and were centrally injected with recombinant rat IL-1β at a dose (2 ng) that decreased social exploration. Only 35F5 was effective in abrogating the behavioral effect of IL-1β. Moreover, 4E2 (1 µg icv) did not potentiate the behavioral response to a subthreshold dose of IL-1β (1 ng icv). To examine the ability of brain IL-1RI to mediate the effects of endogenous IL-1β, mice were centrally treated with 35F5 (4 µg) and peripherally injected with IL-1β (1 µg). Like IL-1 receptor antagonist (4 µg icv), 35F5 abrogated the effects of IL-1β. These results suggest that brain IL-1RI mediates the behavioral effects of IL-1β in mice.

antibodies; social exploration; mouse

INTERLEUKIN-1β (IL-1β) is a proinflammatory cytokine synthesized and released by activated macrophages and monocytes during the host response to infection. Together with other proinflammatory cytokines, it coordinates the immune response to pathogens (9). IL-1β also acts in the brain, where it is synthesized and released by glial cells (2, 14). In the brain, IL-1β is responsible for fever, activation of the hypothalamic-pituitary-adrenal axis, anorexia, sleepiness, and decreased exploration (10). IL-1β binds to two types of receptors belonging to the immunoglobulin superfamily (19) and representing two separate gene products (5). In the immune system, the type I IL-1 receptor (IL-1RI) is predominantly present on T cells and fibroblasts, whereas the type II IL-1 receptor (IL-1RII) is found on B cells and macrophages (19). These two receptors are also expressed in the mouse brain (8, 23, 30), predominantly in the choroid plexus and in the dentate gyrus of the hippocampus. Their cellular localization was demonstrated on neuronal cells of the hippocampus of mice (23, 30) and on human and mouse primary cultured astrocytes (27, 29). IL-1RIs transduce the signal to the nucleus, whereas IL-1RIIs, certainly due to their short intracytoplasmic domain (19), cannot transduce the signal (6). IL-1RIs function then as effectors of IL-1 action, whereas IL-1RIIs have been described to function as inhibitory receptors downregulating IL-1 action (4, 6, 7, 25). This inhibitory function has been demonstrated with in vitro experiments using transfected cells with IL-1RII and showing impaired responses of these cells to an IL-1 stimulation (4, 25). In contrast with these findings, the results of experiments using a monoclonal antibody (MAb) against human IL-1RII (ALVA42) show that IL-1RII mediates some of the effects of IL-1β, including the in vivo inhibition of gastric secretion (21) and stimulation of fever response (16) and the prostaglandin E2 release by hypothalamus explants (20). However, the specificity of binding of ALVA42 has been questioned (11). Moreover, the contribution of IL-1RII to the fever response is doubtful, because there is a positive linear correlation between the affinity of IL-1RII subtype selective ligands and their pyrogenic activity (17).

In view of these contradictory results, the aim of our work was to assess the receptor mechanisms by which IL-1 acts in the brain to induce behavioral alterations, such as decreased social exploration. For this purpose, we used neutralizing MAb raised against IL-1RI and IL-1RII to evaluate the respective role of these receptors in the decrease of social exploration induced by IL-1β. On the basis of previous experiments showing that IL-1 receptor antagonist (IL-1ra), which has a greater affinity for IL-1RI than for IL-1RII in vitro (1), blocks this behavioral effect of IL-1β (3), and despite the fact that this antagonist is usually dose in excess, our first hypothesis was that IL-1RI is the IL-1 receptor subtype mediating decreased social exploration. Our second hypothesis, based on Colotta and colleagues’ reports (6, 7), was that IL-1RII might play a negative regulatory role in the brain. Used as dependent variable, the decrease of social exploration is induced by intracerebroventricular injection of IL-1β (13). We predicted that a MAb raised against IL-1RI should block, whereas a MAb raised against IL-1RII should potentiate, this IL-1β action. The first but not the second hypothesis was supported by the results of the present study.

MATERIAL AND METHODS

Animals

Adult male mice of the CD-1 (ICR) BR strain were obtained from Charles River at 3 wk of age. They were housed in polycarbonate cages (42 × 22 × 17 cm) in groups of 10 until surgery, with food and water freely available, at controlled ambient temperature (22 ± 2°C) and under a 12:12-h light-dark cycle (lights off at 9:00 AM). Experiments were conducted when mice were 8–10 wk old and weighed 25–35 g. A total of 95 mice was used for all the experiments. Juvenile
male mice (21–28 days) from the same strain and raised under the same conditions served as stimulus animals for behavioral observations. The protocol was approved by the Animal Care and Use Committee of the French Minister of Agriculture.

**Surgery**

Mice were anesthetized with a mixture of ketamine (12.2 mg/kg) and xylazine (1.8 mg/kg) intraperitoneally injected (10 ml/kg body wt). Implantation of a guide cannula for intracerebroventricular injections was carried out when mice were 6 wk old. A 23-gauge, 7-mm long, stainless steel guide cannula broventricular injections was carried out when mice were 6 mg/kg) and xylazine (1.8 mg/kg) intraperitoneally injected (10 ml/kg body wt). Implantation of a guide cannula for intracerebroventricular injections was carried out when mice were 6 wk old. A 23-gauge, 7-mm long, stainless steel guide cannula was stereotaxically inserted over the lateral ventricle at the following coordinates from bregma: −0.6 mm anterioposterior, ±1.5 mm lateral, −2 mm vertical according to Lehman's stereotaxical atlas (14a). After surgery, animals were allowed a 2-wk recovery period and individually housed in polypyrilene cages (24 × 14 × 13 cm).

**Behavioral Observations**

All animals were tested (3 observations/animal) the day before the first injection to ensure stability of the behavioral baseline and to familiarize them with the experimental procedure. Behaviorally unstable and aggressive mice were discarded at this stage. The behavioral test took place in the home cage of experimental mice (24 × 14 × 13 cm) during the dark phase of the cycle. The cage was placed in a separate room with a red light, and behavior was monitored via a video camera. A juvenile conspecific was introduced into the cage of the experimental mouse for a period of 4 min, and the observer recorded the amount of time (in s) spent by the adult mouse following, grooming, and sniffing its juvenile conspecific. Social exploration was defined as the total time spent in these behaviors. The first behavioral observation, serving as the baseline value, took place 1 h after lights off. Immediately after, each mouse received its treatments (randomly allocated) and was tested 1.5, 3, and 6 h later (experiments 1 and 2) and 1.5, 3, 6, and 9 h later (experiment 3).

**Reagents**

The IL-1 receptor antagonist (recombinant human IL-1ra; Synergen, Boulder, CO), which binds both IL-1RI and IL-1RII, was diluted in approxyogenic physiological saline (NaCl 0.9%). The dose of IL-1ra was chosen according to previous experiments run in our laboratory (3). The rat anti-mouse IL-1RI MAb (35F5) and the rat anti-mouse IL-1RII MAb (4E2) (Hoffman Laroche, Nutley, NJ) were diluted in PBS (20 mM sodium phosphate, pH 7.4; 0.25 M NaCl). Doses of 35F5 (1 and 4 µg/mouse, experiments 1 and 3) to block brain IL-1RI were selected on the basis of the results of pilot experiments. Because affinity of 4E2 to IL-1RII is twofold higher than 35F5 to IL-1RI (22) and because pilot experiments did not reveal any effect of this MAb at 0.5 µg/mouse, the doses selected were two- to fourfold (experiments 2 and 1, respectively) the dose of 35F5 based on the affinity. Rat immunoglobulin G (IgG) (Sigma) diluted in PBS served as control treatment for the antibodies. IL-1β (recombinant rat IL-1β, NIBSC, Potters Bar, UK) was diluted in a 0.1% bovine serum albumin (BSA) solution (Sigma; A-8806). IL-1β doses were selected from results of pilot experiments. Fresh solutions were made on every day of test.

**Treatments**

Experiment 1. On the test session, mice received a double intracerebroventricular injection (1 µl each) with a 1-min time interval between the two injections. Mice were first treated with four different treatments and their respective controls, which were IL-1ra (2 µg/mouse) vs. NaCl 0.9%, 35F5 (1 µg/mouse) vs. rat IgG (1 µg/mouse), 4E2 (2 µg/mouse) vs. rat IgG (2 µg/mouse), or 35F5 + 4E2 (1 µg + 2 µg/mouse) vs. rat IgG (3 µg/mouse). Mice were then treated with IL-1β (2 ng/mouse) vs. BSA 0.1%. Each mouse was used for two test sessions, receiving the double treatments in a randomized order, with a 3-day interval between two test sessions.

Experiment 2. On the test session, mice received a double intracerebroventricular injection (1 µl each) with a 1-min time interval between the two injections. Mice were first treated with 4E2 (1 µg/mouse) vs. rat IgG (1 µg/mouse) and then treated with IL-1β (1 ng/mouse) vs. BSA 0.1%. Each mouse was used for two test sessions, receiving the double treatments in a randomized order with a 3-day interval between two test sessions.

Experiment 3. On one test session, mice received the first treatment intracerebroventricularly (1 µl/mouse) and the second treatment intraperitoneally (200 µl/mouse) with a 1-min time interval between the two injections. Three different first treatments [rat IgG (1 µg/mouse), IL-1ra (2 µg/mouse), or 35F5 (1 µg/mouse)] combined with two second treatments [IL-1β (1 µg/mouse) or BSA 0.1%] were tested. Each mouse was used for two test sessions with a 3-day interval between two test sessions, receiving two double treatments in such a manner that no mouse received the following first treatment combinations: IL-1ra and IL-1ra, IL-1ra and 35F5, 35F5 and 35F5.

**Statistical Analysis**

Durations of social exploration, expressed as percent of the baseline values, were analyzed according to a three-way analysis of variance (ANOVA) (first treatment × second treatment × time), with repeated measures on time factor in the three experiments. When IL-1ra effects were studied (experiment 1), repeated measures were on second treatment factor. When antibody effects were studied (experiments 1 and 2), repeated measures were on first treatment factor. In experiment 3, first treatment and second treatment factors were independent. If the three-way ANOVA showed a significant interaction between the three factors, data were analyzed according to a one-way ANOVA followed by the post hoc Newman-Keuls test.

### RESULTS

**Experiment 1: Effects of Intracerebroventricular IL-1ra (2 µg / Mouse), 35F5 (1 µg / Mouse), 4E2 (2 µg / Mouse), or 35F5 + 4E2 (1 µg + 2 µg / Mouse) on the Decrease of Social Exploration Induced by Intracerebroventricular IL-1β (2 ng / Mouse)**

To determine which receptor subtype mediates the behavioral effects of IL-1β, mice were first treated with neutralizing antibodies against IL-1RI (35F5) or IL-1RII (4E2), IL-1ra serving as positive control, before receiving the IL-1β injection. IL-1β (2 ng icv) induced a significant decrease of social exploration 1.5 and 3 h after injection (Fig. 1) (second treatment × time interaction in the 4 groups: IL-1ra, F(2,36) = 8.29, P ≤ 0.001; 35F5, F(2,26) = 17.6, P ≤ 0.001; 4E2, F(2,26) = 12.1, P ≤ 0.001; 35F5 + 4E2, F(2,24) = 10.1, P ≤ 0.001). First treatment with IL-1ra (2 µg icv) (Fig. 1A), 35F5 (1 µg icv) (Fig. 1B), or 35F5 + 4E2 (1 + 2 µg icv) (Fig. 1D) abrogated this effect (first treatment × second treat-
Experiment 2: Effects of Intracerebroventricular 4E2 (1 µg/Mouse) on a Subthreshold Dose of Intracerebroventricular IL-1β (1 ng/Mouse) on Social Exploration

To detect a possible negative regulatory role of IL-1RII, mice first treated with 4E2 (1 µg/mouse) were injected with a subthreshold dose of IL-1β (1 ng/mouse). As illustrated in Fig. 2, IL-1β induced a small nonsignificant decrease in social exploration [second treatment × time interaction: F(2,20) = 3.05, P = 0.10] and this effect was not potentiated by first treatment with 4E2 [first treatment × second treatment interaction: F(1,10) = 0.14, P = NS].

Experiment 3: Effects of Intracerebroventricular IL-1ra (4 µg/Mouse) or 35F5 (4 µg/Mouse) on the Decrease of Social Exploration Induced by Intraperitoneal IL-1β (1 µg/Mouse)

To determine if the IL-1 receptors mediating the effects of exogenous IL-1β are the same as those mediating the effects of endogenous IL-1β, mice first treated with an intracerebroventricular injection of IL-1ra or 35F5 were injected with IL-1β in the abdominal cavity. The effect of IL-1β was differentially modulated by the first treatments according to time [first treatment × second treatment × time interaction: F(6,90) = 2.26, P = 0.05]. When mice were first treated with rat IgG (1 µg/mouse) (Fig. 3A), IL-1β (1 µg/mouse) induced a significant decrease of social exploration at all observation times [second treatment effect: 1.5 h, F(1,9) = 18.1, P = 0.01; 3 h, F(1,9) = 8.51, P = 0.05; 6 h, F(1,9) = 17.2, P = 0.01; 9 h, F(1,9) = 6.30, P = 0.05]. When IL-1β injection was preceded by IL-1ra (4 µg/mouse) (Fig. 3B) or by 35F5 (4 µg/mouse) (Fig. 3C), its effects were differentially modulated by the first treatments according to time [first treatment × second treatment × time interaction: F(6,90) = 2.26, P = 0.05].
effects were totally abrogated 3, 6, and 9 h postinjection. At 1.5 h, IL-1β still produced a significant decrease of social exploration [second treatment effect: IL-1ra, F(1,10) = 10.7, P < 0.01; 35F5, F(1,11) = 13.8, P < 0.01], which was significantly attenuated by the first treatment [first treatment effect: F(2,16) = 6.78, P < 0.01; Newman-Keuls: IgG-IL-1β vs. IL-1ra-IL-1β, P < 0.01, and vs. 35F5-IL-1β, P < 0.05].

DISCUSSION

The present results demonstrate that blockade of IL-1RI abrogated the behavioral effects of centrally and peripherally injected IL-1β in mice, whereas blockade of IL-1RII had no effect on centrally injected IL-1β.

Previous in vivo experiments on the role of IL-1 receptor subtypes have mainly been carried out in relationship to the immune effects of IL-1β. Accordingly, blockade of IL-1RI with a neutralizing MAb has been shown to decrease the acute inflammatory response in mice (12, 18, 22), confirming the results from in vitro studies demonstrating the predominant role of IL-1RI in the signal transduction of IL-1. Moreover, peripheral IL-1RI has been shown to be involved in centrally mediated physiological responses to infection: intraperitoneal administration of 35F5 (IL-1RI-neutralizing MAb) abrogated the LPS-induced adrenocorticotrophic hormone release in mice (26); there was a positive correlation between the affinity of selective ligands to IL-1RI intraperitoneally administered and the fever response of rats (17); IL-1RI-knockout mice were found to be resistant to fever, lethargy, and anorexia induced by an intraperitoneal administration of IL-1β (15). Because binding sites to IL-1 are expressed in the brain of mice (8, 23, 30), the aim of this study was to investigate the contribution of the brain type I and type II IL-1 receptors to the central effects of IL-1β on behavior. Our results show that blockade of the brain IL-1RI with a neutralizing MAb (35F5) fully abrogates the decrease of social exploration induced by intracerebroventricularly injected IL-1β, whereas the blockade of brain IL-1RII with a neutralizing MAb (4E2) has no effect. Moreover, the simultaneous blockade of the two IL-1 receptor subtypes produces an identical curve response as blockade of IL-1RI alone, confirming that IL-1RI plays a predominant role in the IL-1β-induced decrease of social exploration. This work is the first to demonstrate the functionality of IL-1RI subtype in the brain of mice.
The significance of these results is critically dependent on the effectiveness and specificity of the MAbs. 35F5 and 4E2 have been shown to bind specifically to IL-1RI and IL-1RII, respectively, without any interference (22). The affinity of 4E2 to IL-1RII, twofold greater than 35F5 to IL-1RI (22), ensures the full blockade of brain IL-1RII by the doses used in this study. The lack of effect of nonspecific rat IgG injected in control animals confirms the specificity of both MAbs effects.

The fact that IL-1RI blockade abrogates the behavioral effect of IL-1β does not allow the exclusion of a possible contribution of IL-1RII to the regulation of IL-1 action in the brain. In the immune system, IL-1RII is described as an active regulatory target that is able to downregulate IL-1 action (4, 6, 7, 25). Because IL-1β has a higher affinity for IL-1RII than for IL-1RI (28), this property should be of physiological importance. In accordance with this hypothesis, blockade of IL-1RII with a MAAb was found to potentiate the activity of a suboptimal concentration of IL-1β on cytokine production by cultured monocytes (6), and transfection of cells with IL-1RII impaired their responsiveness to IL-1 (4, 25). In contrast with these results, blockade of IL-1RII in the brain with a neutralizing MAAb (ALVA42) abrogated the in vivo fever response of rats to IL-1β (16) and the ex vivo IL-1β-induced prostaglandin E₂ release from hypothalamic explants (20). However, the significance of these results is doubtful because ALVA42 was subsequently found to bind the human leukocyte antigen DR α- and β-chains rather than IL-1RII (11). Therefore, we tested the possible negative regulatory role of IL-1RII in the brain by looking for a possible potentiation of the effect of a subthreshold dose of intracerebroventricular IL-1β on social exploration in mice treated with a neutralizing MAAb against IL-1RII (4E2). We were unable to observe any effect of this MAAb on the behavioral effects of a subthreshold dose of IL-1β. It is difficult to decide whether this negative result is due to the lack of sensitivity of the behavioral response under study or to the lack of a functional role of IL-1RII in the brain.

Because all these results were observed with exogenous IL-1β, it was important to study the physiological role of brain IL-1RI on the action of endogenous IL-1β. A convenient way to induce the expression of brain IL-1β is to inject IL-1β at the periphery (24). We therefore chose this model to induce endogenous brain IL-1β. Our results show that blockade of the brain IL-1RII with a neutralizing MAAb (35F5) abrogated the decrease of social exploration induced by intraperitoneally injected IL-1β. The fact that this abrogation was only partial 1.5 h after the injection suggests that a minor part of the decrease of social exploration was due to the IL-1β action at the periphery or to other proinflammatory cytokines (e.g., tumor necrosis factor-α and IL-6) that could be induced in the brain in response to peripheral IL-1β. This cannot be explained by interaction of IL-1β with brain IL-1RII, because the blockade of central IL-1 receptors with IL-1ra also partially abrogated the IL-1β effects on behavior 1.5 h postinjection. These results confirm that IL-1β acts in the brain to decrease social exploration in mice (3) and clearly demonstrate that these effects are mediated by brain IL-1RI.

Perspectives

The existence of multiple cytokine receptor subtypes in the brain raises questions about the functional significance of this diversity. The present results clearly show the importance of selecting appropriate tools to address these questions.

We thank V. Tridon for skillful assistance with surgery, Dr. R. Chizzonite for the generous gift of rat anti-mouse IL-1 receptor MAAb 35F5 and 4E2, and Dr. R. J. Vannice for the generous gift of recombinant human IL-1ra. Recombinant rat IL-1β was obtained through the BiOMED 1 Concerted Action “Cytokines in the Brain.” This work was partially supported by National Institutes of Health Grant MH-51569-01A2 to K. W. Kelley. S. Cremona is financially supported by the Conseil Régional d’ Aquitaine (France). Address for reprint requests: S. Cremona, Inserm U394 Neuroimmunologie Intégrative, 1 rue Camille Saint-Saëns, 33077 Bordeaux Cedex, France.

Received 20 June 1997; accepted in final form 17 November 1997.

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