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FULL PAPER

Brain interleukin-1 is involved in blood interleukin-6 response to immobilization stress in rats

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Abstract

Interleukin (IL)-6, a cytokine for host defense responses to infection and imflammation, is known to be induced by non-invasive physical or psychological stress, too. To test possible involvement of brain IL-1 in the stress-induced IL-6 production, IL-1 mRNA expression in the hypothalamus, in parallel with blood IL-6 level, was examined in rats subjected to restriction of their movement (immobilization stress). When rats were immobilized, the hypothalamic IL-1 β mRNA level was increased in 1 hr, followed by progressive rises in the serum IL-6 level. The immobilization-induced rise in serum IL-6 was mimicked by intracerebroventricular (icv) administration of IL-1 β under normal conditions, whereas it was attenuated by icv injection of an IL-1 receptor antagonist. These results indicate that IL-1 in the hypothalamus plays a pivotal mediating role in the stress-induced peripheral IL-6 production.

Key words : interleukin-1, interleukin-6, brain, stress

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Introduction

Interleukin (IL)-6 is one of the pivotal cytokines for host defense responses to infection and inflammation. It is known that non-invasive stress also increases peripheral IL-6 production, independently of endotoxemia, tissue damage and inflammation. For example, when animals were subjected to restriction of their movement (immobilization stress), the blood IL-6 level rises in 1 hr, following increased expression of IL-6 mRNA in the liver and spleen (6). Takaki et al. (14, 15) reported that the stress-induced blood IL-6 response is attenuated by either partial hepatectomy or surgical severing of the hepatic sympathetic nerves, but not by splenectomy. Since IL-6 mRNA expression is also elevated by catecholamines in primary cultured hapatocytes (9), the stress-induced blood IL-6 response would be largely due to sympatho-adrenomedullarly mediated activation of IL-6 synthesis in the liver.

In parallel with peripheral IL-6, production and release of IL-1 in the hypothalamus is also increased after immobilization stress (11, 13). Moreover, intracerebroventricular (icv) administration of minute amounts of IL-1 is known to activate the sympathetic nervous system and to induce peripheral IL-6 (7, 8, 16). These previous results collectively suggest the involvement of hypothalamic IL-1 in the stress-induced periperal IL-6 responses. To test this idea, in the present study, we examined 1) IL-1 mRNA expression in the hypothalamus, in parallel with blood IL-6 level, in response to immobilization stress in rats, and 2) the effects of icv injection of IL-1 receptor antagonist (IL-1 Ra) on the blood IL-6 response.

Materials and Methods

Experimental animals

Male Wistar rats (9-10 weeks old) were housed in plastic cages at 24 ± 1 ℃ with a 12-hr light-dark cycle (lights on at 7 am) and given free access to laboratory chow and water. Some rats were anesthetized by sodium pentobarbital (45 mg/kg, ip), and were stereotaxically implanted with a stainless steel cannula unilaterally into the left cerebral ventricle (0.9 mm posterior to the bregma, 1.7 mm lateral from the midline, 3.3 mm below the dura), according to the stereotaxic coordinates adopted from the brain atlas of Pellegrino et al. (12). After a recovery period of 10-14 days, they were used for experiments. The experimental procedure and care of animals were in accordance with the guidelines of the Animal Care and Use Committee of Hokkaido University.

Cytokines

Human recommbinant IL-β (2 x 10^7U/mg protein) was kindly provided by Dr. Y. Hirai (Otsuka Pharmaceutical, Tokushima, Japan). IL-1Ra was purchased from Pepro Tech EC (London, UK). Heat inactivated IL-1Ra was prepared by heating IL-1Ra at 100℃ for 2 hr. These were diluted with sterilized 20mM phosphate-buffered saline (PBS) and injected into the cerebroventricle at a volume of 5 μl.

Immobilization stress and icv injection

Immobilization of rats was performed by restraining them in the supine position by taping their limbs and trunk to a board. After immobilization for 0, 1, 2 and 4 hr, rats were decapitated and blood was collected. Whole brain was also taken and the hypothalamus was isolated according to the method of Glominski et al. (3). In another series of experiment, rats were given an icv injection of 200 ng human recombinant IL-1β, instead of immobilization, and decapitated as described

above. Some rats were given an icv injection of 1 μg IL-1Ra 15 min before immobilization.

Assay of serum IL-6

The concentration of serum IL-6 was measured using an IL-6-dependent cell line MH60.BSF2 (a gift from Dr. T. Matsuda, Osaka University, Suita, Japan) as described previously (10). The minimum concentration of serum IL-6 assayed by this method was 2×10^{-2} U/ml (4 pg/ml).

RT-PCR and Southern blot analysis of IL-1 mRNA

Total RNA was extracted from hypothalamic tissue samples by the guanidine isothiocyanate method using TRIzol solution (Gibco BRL, Gaithersburg, MD, USA). IL-1β mRNA was assayed by the combination of reverse transcription (RT)-polymerase chain reaction (PCR) and Sourthern blotting. Briefly, total RNA (1 μg) was denatured at 70℃ for 10 min, cooled immediately on ice, and then reverse-transcribed using 10 U/ml of Moloney murine leukemia virus reverse transcriptase (Gibco), 100 pM poly (dT) primer and 20 μM dNTPs in a total volume of 10 ml at 37℃ for 1 hr. After heating at 95℃ for 5 min, PCR amplification of the RT product (1 μl) was performed with 25 U/ml Taq DNA polymerase (Perkin Elmer, Norwalk, CN, USA), 20 μM dNTPs, 1.5 mM $MgCl_2$ and 0.5 μM primers for rat IL-1β in a total volume of 50 μl. The forward and reverse primers used were 5'-TTGTTATGGGTTTCTTCTTCTACCT-3' and 5'-GAAGAAGTTCTACTTCCTTTTCTT-3', respectively. PCR was conducted for 33 cyles consisting of denaturation at 94℃ for 30 sec, annealing at 58℃ for 30 sec, and DNA extension at 72℃ for 30 sec. The PCR product was electrophoresed on a 2 % agarose gel, stained with ethidium bromide, and then transferred to a nylon membrane (Amersham, Buckinghamshire, UK). A cDNA probe corresponding to nucleotides −2 to +378 bp of the published cDNA sequence of rat IL-1β was synthesized by RT-PCR using total RNA extracted from rat spleen, and labelled with [α-^{32}P] dCTP using a multiprime DNA labeling kit (Amersham). The membrane was hybridized to the labelled probe at 65℃ for 20 hr in the presence of 0.2 mg/ml salmon sperm DNA (Sigma, St. Louis, MO, USA). After washing the membrane, the radioactivity present on the membrane was measured with a bioimage analyzer (BAS1000, Fuji Film, Tokyo, Japan), and depicted on X-ray films. mRNA of glyceraldehyde 3-phosphate dehydrogenase (G3PDH) was also analyzed by RT-PCR and Sourthern blotting as described previously (9).

Data analysis

Data were expressed as means ± SEM. Statistical comparison was made by analysis of variance, followed by Scheffe's F test.

Results

Blood IL-6 responses to immobilization and icv injection of IL-1

The IL-6 level in the blood is known to be elevated not only during inflammation, but also during non-invasive stress such as immobilization and open field tasks (5, 6, 14). We have reported that intracranial administration of IL-1 also induced peripheral IL-6 production (7, 8). To compare the IL-6 responses to non-invasive stress and central IL-1, first, we examined the time-course of blood IL-6 responses to immobilization stress and icv injection of IL-1β in rats. As shown in Fig. 1, the serum IL-6 level was 0.61 ± 0.11 U/ml in untreated control rats (0 hr), but after immobilization it increased rapidly to reach a peak of 5.14 ± 2.11 U/ml at 2 hr, and returned to a lower level in 4 hr. A quite similar time-

dependent response was found when a minute amount (200 ng/rat) of IL-1β was injected into the cerebral ventricle : that is, a transient increase of serum IL-6 at 2 hr and a decrease at 4 hr. Thus, the immobilization-induced IL-6 response was mimicked by icv injection of IL-1.

Hypothalamic IL-1 response to immobilization

The protein and mRNA levels of IL-1 were reported to increase in the hypothalamus of immobilized rats (11, 13). To confirm these previous results, we examined hypothalamic IL-1β mRNA expression in the same rats used in Fig. 1. In preliminary experiments, we tried to assay IL-1β mRNA by the conventional Northern blot analysis, but failed to detect any signal in our samples, probably due to low expression levels in the hypothalamus. Therefore, we assayed it by combination of RT-PCR and Southern blotting. For qunatitative estimation of IL-1β mRNA, the appropriate number of cycles (33 cycles) and the amount of total RNA in the samples were determined to confirm the linearity of the RT-PCR assay. In addition, G3PDH mRNA was also analyzed by RT-PCR and Southern blotting, and the level of IL-1β mRNA was expressed relative to that of G3PDH mRNA. As shown in Fig. 2, the IL-1β mRNA level in the hypothalamus increased rapidly after immobilization, reached a peak at 1 hr and returned to lower levels at 2 hr and thereafter.

Effects of icv injection of IL-1Ra

To clarify the possible mediating role of brain IL-1 in immobilization-induced peripheral IL-6 production, rats were given icv injection of IL-1Ra, and 15 min later they were immobilized for 2 hr. As shown in Fig. 3, in rats injected with heat-inactivated IL-1Ra immobilization rose the serum IL-6 level to almost

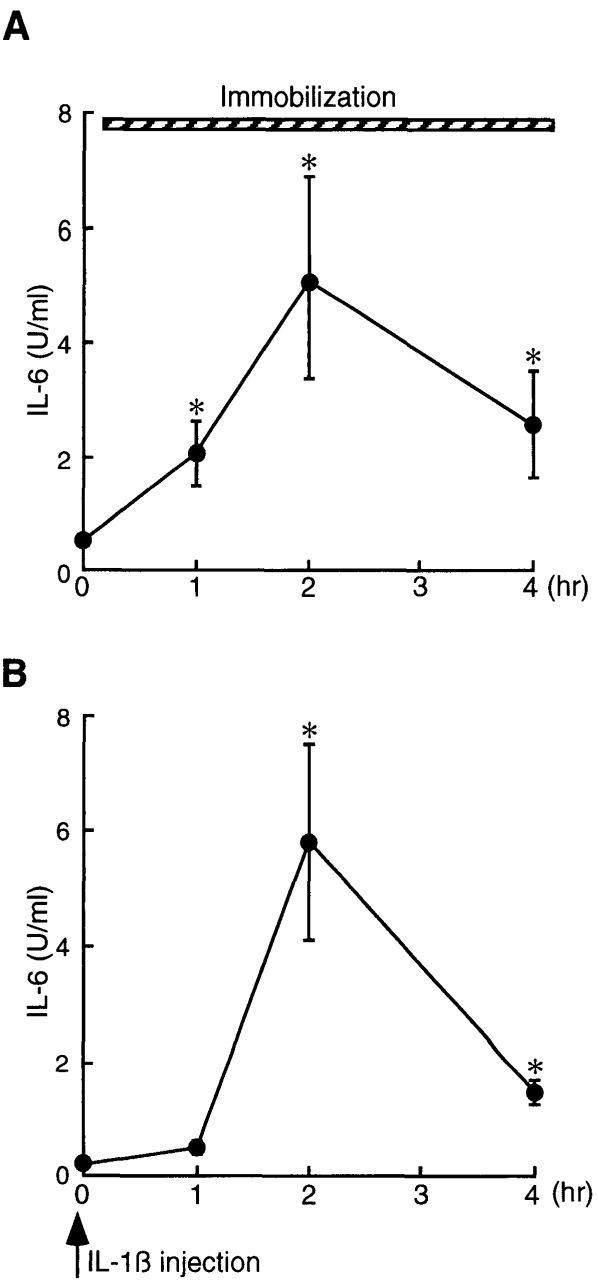

Fig. 1. Blood IL-6 responses to immobilization and icv injection of IL-1.
Rats were immobilized (A) or given icv injection of 200 ng IL-1β (B), and serum IL-6 levels were measured at 0, 1, 2, and 4 hr. Values are means ± SEM for 4 - 7 rats. *P<0.05 vs. the 0-hr value.

the same level as those seen in intact rats (see Fig. 1). However, in rats injected with IL-1 Ra the IL-6 response was reduced by 69%.

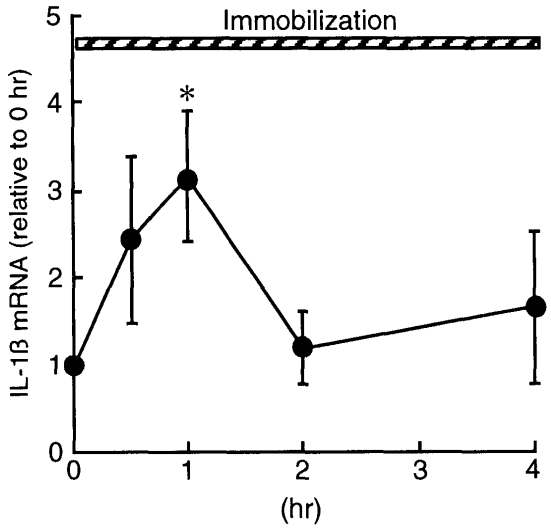

Fig. 2. Hypothalamic IL-1β mRNA responses to immobilization.
The IL-1β mRNA levels were normalized to those of G3PDH, and expressed relative to the 0-hr value. Values are means ± SEM for 3 rats. *P<0.05 vs. the 0 -hr value.

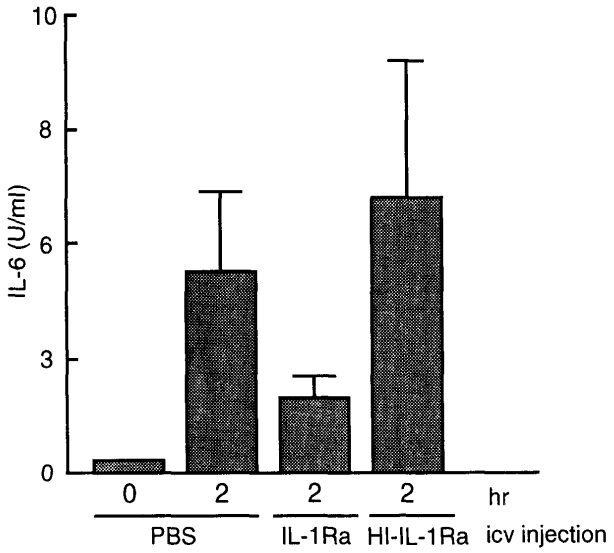

Fig. 3. Effects of icv injection of IL-1Ra on the immobilization-induced blood IL-6 response.
Rats were given icv injection (1μg/rat) of intact or heat-inactivated IL-1Ra (HI-IL-1Ra), and 15 min later they were immobilized for 2 hr. Values are means ± SEM for 3 - 5 rats.

Thus, the peripheral IL- 6 response to immobilization was much attenuated by blocking the actions of brain IL-1.

Discussion

In the present study, we showed that when rats were subjected to immobilization stress, the hypothalamic IL-1 mRNA level was increased rapidly, followed by substantial rises in the serum IL-6 level. The immobilization-induced rise in serum IL-6 was mimicked by icv administration of IL-1, whereas it was attenuated by icv injection of an IL-1 receptor antagonist.

There have been reports that icv injection of minute amounts of IL-1 produces a rise in the blood IL-6 level (2, 7). Being consistent with these previous findings, we demonstrated that the serum IL-6 response to icv injection of IL-1 follows a quite similar time-course to those to immobilization, showing a rapid rise at 1 - 2 hr and a subsequent decline. Moreover, it is known that both responses of serum IL-6 are largely based on the same peripheral mechanism, namely, the sympatho-adrenomedullarly mediated activation of IL-6 synthesis in the liver (7, 14). These facts collectively suggest that the factors and/or mechanisms in the brain are similar in the IL -6 responses to these two challenges. One of the simplest idea may be that IL-1 in the brain is involved in the immobilization-induced IL-6 response. This seems to be supported by the present finding that the IL-1 mRNA level in the hypothalamus was increased after immobilization, but just prior to the rise in the serum IL-6 level. More direct evidence for the above idea was obtained from the findings that the immobilization-induced IL-6 response was much attenuated when the actions of central IL-1 was blocked by icv injection of IL-1Ra. The inhibitory effect of IL-1 Ra administrated into the hypothlamus was

also documented on the adrenocortical activation and intrahypothalamic norepinephrine release induced by immobilization (13). Thus, IL-1 in the hypothalamus is likely to play a critical mediating role in the various responses to non-invasive stress, including the adrenocortical and sympatho-adrenomedullar activation.

The origin and action site of IL-1 in the hypothalamus are not well identified, but there have been many reports that glial cells can induce IL-1 synthesis in response to various physiological and pathological stimuli (13). In addition, IL-1 is expressed in brain neurons under nonstimulated conditions. IL-1 immunoreactive nerve fibers are located in several hypothalamic areas, such as the ventromedial hypothalamic nucleus, the posterior hypothalamus and the region of the hypophysial vessels of the median eminence (1). There has also been evidence for the existence of IL-1 receptor in the hypotalamus. For example, Hammond et al. (4) demonstrated the type I IL-1 transcript and the protein are expressed in astrocytes of the human hypothalamus. All these findigs imply the role of brain IL-1 as a neurotransmitter/neuromodulator, although not yet been established, under both inflammatory and non-inflammatory stress conditions.

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