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Interleukin-2: Structural and Biological Relatedness to Opioid Peptides

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Key Words

Interleukin-2 · Cytokines · Site-directed mutagenesis · Domain · Pain threshold · Opioid receptors · Neuroregulatory

Abstract

Interleukin (IL)-2 is not only an immunoregulatory factor, but also an analgesic molecule. There are distinct domains of immune and analgesic functions in the IL-2 molecule. The analgesic domain is located around the 45th Tyr residue of human IL-2 in tertiary structure. Antiopioid (β-endorphin, Leu-enkephalin, Met-enkephalin and dynorphin A1–13) sera partially neutralized the analgesic activity of IL-2. Monoclonal antibody against the IL-2 receptor α subunit (Tac) could not block the analgesic activity of IL-2. There existed cross-reactivity between IL-2 and antiopioid sera by indirect ELISA. These studies show strong structural and biological similarities between IL-2 and opioid peptides. The tertiary structure around the 45th residue of IL-2 composes the analgesic domain that is similar to that of endogenous opioids. These results are consistent with the hypothesis that multiple domains of cytokines serve as the structural bases for the immunoregulatory and neuroregulatory effects of cytokines.

Introduction

There is now overwhelming evidence that communication occurs between the immune system and the central nervous system (CNS), representing an important homeostatic mechanism in the body. Cytokines play a crucial role in this communication and exert powerful effects on the brain. Thus, interleukin (IL)-2 is not only an important immunoregulatory molecule that enhances T lymphocyte proliferation after antigenic stimulation, but is also an important neuroregulatory molecule in the CNS [1, 2]. Investigations in vivo and in vitro have elucidated the following facts: IL-2 and IL-2 receptor (IL-2R) have been detected in the brain [3]; IL-2 supports the survival of neurons cultured from various regions of the brain [4], stimulates oligodendrocyte proliferation and maturation [5], affects the hypothalamic-pituitary function [6] and produces behavioral and electrocorticogram spectrum changes [7].

We have previously reported that IL-2 has an analgesic effect in both the CNS and peripheral nervous system (PNS) [8, 9]. The present experiment provided further information on the analgesic effect of IL-2 and on its structural and biological relatedness to opioid peptides.

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Materials and Methods

Preparation of IL-2 and Its Mutants

The procedure of mutagenesis of the human IL-2 gene used was that described by Kunkel [10]. Bacteriophage M13mp19-IL-2 was propagated in *Escherichia coli* CJ236 to produce uracil-containing DNA (U-DNA), and single-strand U-DNA was prepared. One microgram of U-DNA was mixed with 1 pmol of 5′-phosphorylated synthetic oligonucleotide to hybridize and synthesize the complementary strand. Then the mixture was used to transform the competent *E. coli* JM105. The single-strand DNA from the colonies was sequenced using the dideoxy chain termination method. The mutated gene in the double-strand DNA of M13 was released by *Eco*RI and *Sal*I digestion and ligated to the expression vector pLy-4.

The purification of IL-2 proteins was directed by Hua Xin High Biotech Inc., where human recombinant IL-2 for clinical usage is produced. The fermentation of *E. coli* JF1125, harboring normal or various mutated IL-2 genes, was performed in M9 medium at 30°C to early logarithmic phase. The cultures were then transferred to 42°C to express the proteins for 3 h. The bacteria were harvested by centrifugation. IL-2 and its analog protein were purified by the collection of the IL-2 inclusion body and Sephacryl S-200 chromatography. In brief, bacteria were suspended in phosphate buffer solution and sonicated. The sonicated suspension was then centrifuged at 8,000 rpm for 10 min to obtain the inclusion body pellet. The inclusion body pellet was dissolved in 2% sodium dodecyl sulfate (SDS), 100 m*M* ammonium acetate (pH 7.0) and 4 m*M* 2-ME, and loaded onto a Sephacryl S-200 column with the same solvent as the elution buffer. The sample was collected and renatured, and then centrifuged at 0°C at 15,000 rpm to remove the SDS, and aliquoted for lyophilization.

Immune Bioactivity Assay of IL-2

The immunoactivity of IL-2 was determined according to the method introduced by Stern et al. [11], which utilized the IL-2-dependent murine cell line CTLL-2. All bioassays were quantitated with IL-2 from Boehringer Mannheim as a standard.

Animals and Surgery

Adult male Sprague-Dawley rats (160–180 g) were used. Throughout the study, the animals were maintained on a 12-hour light/dark cycle. The animals were stereotaxically implanted with stainless steel guide cannulae, under pentobarbital anaesthesia (35 mg/kg, i.p.), to permit intracerebroventricular infusion. After surgery, a minimum of 72 h was allowed for recovery before experiments were carried out.

Analgesia Assay

The potassium iontophoresis-induced tail-flick was used to measure the pain threshold (PT) of the rats [12]. We took the mean value of three intensities of the current (mA; every 1 min) at the moment of response as the PT. The intensity of the current was measured 10 min after IL-2 (120 pmol) was injected into the lateral ventricle in a volume of 8 µl and every 20 min. The percentage of the PT changes was calculated on the basis of the mean value of three PTs before IL-2 injection. Antiopioid sera against β-endorphin (AEPS), Leu-enkephalin (ALEKS), Met-enkephalin (AMEKS) and dynorphin A1–13 (ADYNS) (Peninsula Lab Inc.), and monoclonal antibody (McAb) against IL-2R (Tac) (Biotinge Biomedicine Co.) in a volume of 5 µl were injected 1 h before the IL-2 injection. Between 8 and 12 male Sprague-Dawley rats were used in each group. The data are expressed as mean ± SD. Statistical analysis was conducted with Student's test.

Indirect ELISA

Indirect ELISA was used to measure the cross-reactivity between IL-2 and antiopioid sera, according to the method of Brown et al. [13]. Flat-bottomed 96-well microtitre plates were coated with 100 µl of 50 m*M* sodium carbonate buffer (pH 9.6) containing 500 ng of IL-2 at 4°C overnight. The plates were washed with phosphate-buffered saline (PBS)/0.05% Tween 20 (PBS-T) and blocked with 100 µl of 1% bovine serum albumin in PB/0.05% Tween 20 (PBT) for 1 h at 37°C. After the plates were washed with PBS-T, antiopioid sera were diluted with PBT in a volume of 100 µl and added to each well (anti-IL-2 McAb as positive control) for 2 h at 37°C. The plates were washed with PBT and horseradish-peroxidase-labeled IgG in 100 µl of PBT was added to each well and the plates incubated for 1 h at 37°C. The plates were again thoroughly washed and the reaction was measured after addition of the substrate, 50 µl of 3,3′,5,5′-tetramethylbenzidine (Kirkegaard & Perry Lab Inc.), for 15 min in the dark at room temperature. The reaction was stopped by the addition of 50 µl of 2 *M* H_2SO_4, and absorbance values measured at 450 nm using an automatic microplate reader (Bio-Rad).

Results

Immunoactivity of IL-2 and Its Mutants

Two IL-2 mutants, 20Leu-IL-2 and 45Val-IL-2, were obtained by using oligonucleotide site-directed mutagenesis (purity >95%). After the 20th Asp residue of human IL-2 was mutated to Leu, the immunoactivity of the mutant (20Leu-IL-2) was lost completely (up to 4,000 n*M*, the immunoactivity still cannot be detected). However, IL-2 still maintained strong immunoactivity (2,490,000 U/mg), becoming 90.9% of that of the wild-type IL-2 (2,740,000 U/mg) after the 45th Tyr residue was changed to a Val residue.

Analgesic Effect of IL-2 and Its Mutants in the CNS

IL-2 had a significant analgesic action in the CNS. The PT was increased significantly after IL-2 injection (120 pmol). The analgesic effect was shown significantly 10 min after IL-2 injection and reached a maximum 50 min after IL-2 injection. Figure 1 shows the analgesic activity 50 min after IL-2 injection (120 pmol) ($p < 0.01$: IL-2 vs. control). 20Leu-IL-2 still maintained strong analgesic activity ($p > 0.05$: 20Leu-IL-2 vs. IL-2), but the analgesic activity of 45Val-IL-2 disappeared. Pretreatment with the McAb against IL-2R (Tac) did not influence the PT changes induced by IL-2 injection. There was no significant difference between the IL-2 group and the IL-2 + McAb group (fig. 1). However, antiopioid sera were able to partly block the PT increase after IL-2 injec-

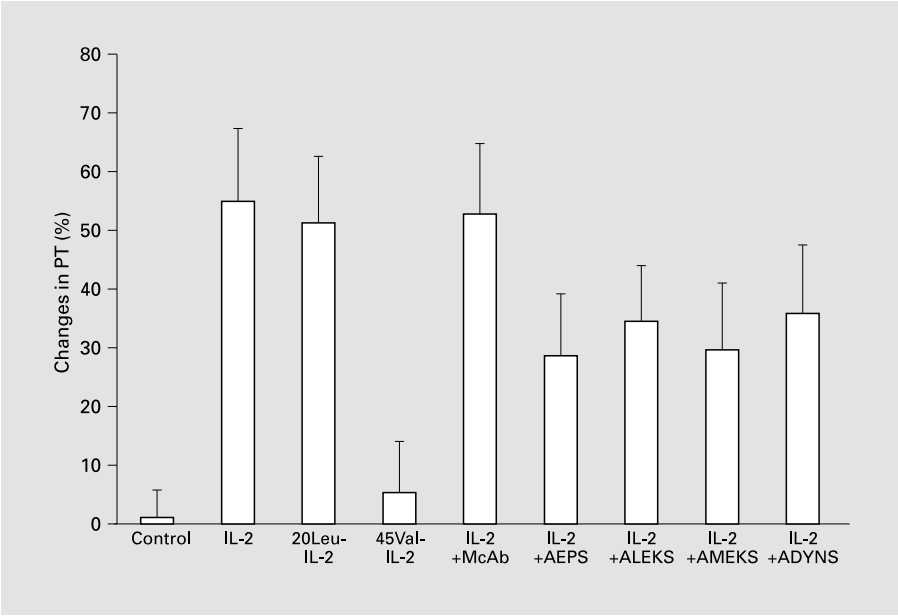

Fig. 1. Changes in PT 50 min after the injection of IL-2 and its mutants (120 pmol). The influence of antiopioid sera and McAb against IL-2R (Tac) on the analgesic effect of IL-2 is also shown. The PT of the rats was increased after injecting IL-2 into the lateral ventricle ($p < 0.01$: IL-2 vs. control). This effect could not be blocked by pretreatment with McAb against IL-2R (Tac) (1:20 dilution) ($p > 0.05$: IL-2 + McAb vs. IL-2). Antiopioid sera (1:20 dilution) partially neutralized the analgesic activity of IL-2 ($p < 0.05$: IL-2 + ALEKS and IL-2 + ADYNS vs. IL-2; $p < 0.01$: IL-2 + AEPS and IL-2 + AMEKS vs. IL-2). Although the immunoactivity of the IL-2 mutant 20Leu-IL-2 disappeared, the analgesic activity remained ($p > 0.05$: 20Leu-IL-2 vs. IL-2). However, the analgesic activity of the IL-2 mutant 45Val-IL-2 was lost completely ($p > 0.05$: 45Val-IL-2 vs. control), although strong immunoactivity remained.

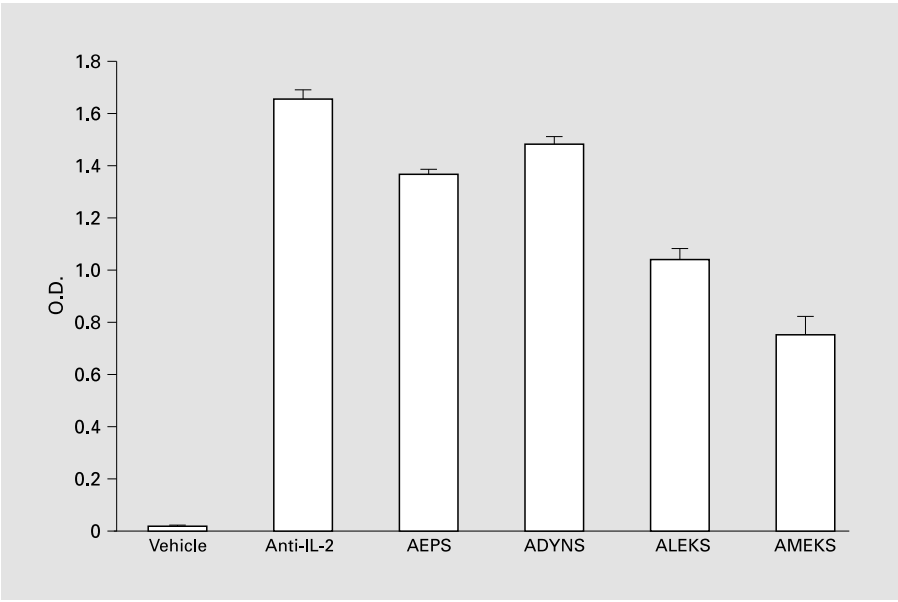

Fig. 2. Cross-reactivity between IL-2 and antiopioid sera. There were significant cross-reactivities between IL-2 and the four antiopioid sera AEPS (1:1,800), ADYNS (1:1,000), ALEKS (1:1,500) and AMEKS (1:2,000) ($p < 0.01$ vs. vehicle). Anti-IL-2 = McAb against IL-2 as positive control; O.D. = optical density.

tion ($p < 0.05$: IL-2 + ALEKS and IL-2 + ADYNS vs. IL-2; $p < 0.01$: IL-2 + AEPS and IL-2 + AMEKS vs. IL-2) (fig. 1).

Cross-Reactivity between IL-2 and Antiopioid Sera

There existed significant cross-reactivity between IL-2 and four antiopioid sera (AEPS, ALEKS, AMEKS and ADYNAS) by indirect ELISA (fig. 2), suggesting that there are common antigenic determinants between IL-2 and endogenous opioid peptides (EOPs).

Discussion

It has been reported that IL-2 has an antinociceptive effect in both the CNS and PNS [8, 9]. Our experiments provide further information on the opioid-like activity of IL-2 in the CNS, as demonstrated by the iontophoresis-induced tail-flick method.

The analgesic or antinociceptive effect of IL-2, in both the CNS and PNS, can be blocked or reversed by naloxone, an opioid receptor antagonist [8, 9]. This suggests that the analgesic effect of IL-2 may be related to the

opioid receptors. There are two possible mechanisms for the blockage of the analgesic effect of IL-2 by naloxone. Firstly, after having bound to the IL-2 high-affinity receptor (consisting of α, β and γ subunits), IL-2 may indirectly induce an increase in the concentration of EOPs, which in turn act on opioid receptors. Secondly, IL-2 may interact directly with opioid receptors to elicit the analgesic effect in the CNS.

Antiopioid sera partially neutralized the analgesic activity of IL-2. This result seems to support the first possibility. However, the McAb against IL-2R (Tac) could not block the analgesic effect of IL-2, excluding the possibility of IL-2 binding directly to the α subunit of IL-2R. It has been reported that the 20th Asp residue of the human IL-2 molecule is crucial for its binding to the IL-2R subunit β, and for inducing the corresponding intracellular signal transduction and immunoactivity [14]. After the 20th Asp residue of IL-2 was mutated to Leu, IL-2 could not bind to the β subunit of IL-2R, but it was still possible to increase the PT of the rats, suggesting that the analgesic effect is not mediated through the β subunit of IL-2R. Moreover, human IL-2 has been shown to be able to bind to the opioid receptor and induce corresponding signal transduction in both NG108-15 cells, which express δ opioid receptor uniformly, and HEK293 cells transfected with the δ opioid receptor gene [9]. Therefore, it is suggested that the analgesic effect of IL-2 may be mediated by IL-2 interaction with the opioid receptor. Our observation that antiopioid sera partially neutralized the analgesic activity of IL-2 suggests that there exist structural similarities between the human IL-2 molecule and opioid peptides. From this finding, it is inferred that the antiopioid sera may block the analgesic domain of IL-2, which is similar to that of opioid peptides, and thus neutralize the analgesic activity of IL-2.

A common characteristic of EOP molecules is the Tyr-Gly-Gly-Phe sequence in the amino termini, among which the aromatic residues, Tyr and Phe, are crucial for the analgesic activity of EOPs. Although there is no similar sequence of Tyr-Gly-Gly-Phe in the IL-2 primary structure, there is a Tyr-×-×-Phe structure similar to that of EOPs around the 45th Tyr residue in its tertiary structure of IL-2 [15, 16]. After the 45th Tyr residue was changed to Val using site-directed mutagenesis, the IL-2 mutant lost its analgesic activity completely, but kept its immunoactivity. Above all, it seems that there exist distinct domains in the IL-2 molecule that mediate immune and analgesic activity, respectively. It is suggested that the structure is similar in the IL-2 molecule to the active site of opioid peptides.

There were significant cross-reactivities between IL-2 and the four antiopioid sera. These studies revealed a strong antigenic relatedness between the human IL-2 molecule and opioid peptides, implying that there are underlying structural similarities.

Antiopioid sera cannot block the immunoactivity of IL-2 [unpubl. data], but can block the analgesic effect of IL-2, further suggesting distinct domains mediating the immune and analgesic effects of IL-2 and structural relatedness between IL-2 and opioid peptides.

Both structural and functional similarities have been demonstrated between human leukocyte interferon (INF-α) and endorphins [17, unpubl. data]. We have proposed that the multiple domains in cytokines serve as the structural basis for the immunoregulatory and neuroregulatory effects of cytokines. Thus, multiple actions of cytokines might be mediated by distinct domains or functional sites interacting with different receptors or receptor subtypes [18]. The strong structural and biological similarities between IL-2 and opioid peptides shown in these experiments are consistent with the above hypothesis.

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