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INTERLEUKIN-1β ANALOGUES WITH MARKEDLY REDUCED PYROGENIC ACTIVITY CAN STIMULATE SECRETION OF ADRENOCORTICOTROPIC HORMONE IN RATS

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SUMMARY: We examined the adrenocorticotropic hormone-releasing activities of several human interleukin-1β analogues that have markedly reduced pyrogenic activities in rats. Among the analogues tested, [Gly^{4}]-, [Leu^{93}]- and [1-148]-interleukin-1β increased the plasma adrenocorticotropic hormone level to almost that induced by authentic human interleukin-1β. Modifications of the N-terminus of the authentic molecule, i.e., [7-153]- and [Des-Ala^{1}, Asp^{4}]-interleukin-1β, significantly reduced the hormone-releasing activity. These data suggest that the adrenocorticotropic hormone-releasing activity of human interleukin-1β resides in the N-terminal structure of the authentic peptide and can be separated from its pyrogenic activity.

IL-1β, a polypeptide originally found in activated macrophages, has been proposed to exert a wide range of biological activities including neuroendocrinological effects during immune and inflammatory responses (1,2). Although some attempts have been made to investigate the active domains of the IL-1 molecule responsible for its various immunological and inflammatory activities

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Abbreviations: hIL-1β, human interleukin-1β; ACTH, adrenocorticotropic hormone; RIA, radioimmunoassay.

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(3,4), there have been no reports about the structure-function relationship of the neuroendocrinological activity of the IL-1 molecule. Therefore, we compared the ACTH-releasing activities with the pyrogenic activities of authentic hIL-1β ([1-153]) and its analogues produced by making the following amino acid modifications: substitution of glycine ([Gly^4]IL-1β) for Arg^4, substitution of leucine ([Leu^{93}]IL-1β) for Lys^{93}, deletion of Ala^1 and substitution of aspartic acid ([Des-Ala^1, Asp^4]IL-1β) for Arg^4, and deletion at the N-terminus ([7-153]IL-1β), and deletion of the C-terminus ([1-148]IL-1β). All of these analogues were synthesized by site-specific mutagenesis technology.

MATERIALS AND METHODS

Materials and experimental protocol

Adult male rats of the Wistar strain, each weighing 300-350g, were kept under a controlled temperature (25 ± 1 °C) and fixed light-dark schedule (lights on at 0600 h, off at 1800 h). Food and water were provided ad libitum. A Silastic cannula was inserted through the jugular vein into the right atrium several days before the blood sampling.

At least 12 hours prior to blood sampling, which routinely began at 0945 h, cannulated animals were moved to a special sampling cage in which blood could be drawn from the rats under conscious, freely-moving conditions without apparent stress throughout the experimental period (5). All of the polypeptides tested, hIL-1β, [Gly^4]IL-1β, [Leu^{93}]IL-1β, [Des-Ala^1, Asp^4]IL-1β, [7-153]IL-1β and [1-148]IL-1β, were synthesized by recombinant DNA technology with or without site-specific mutagenesis (6,7). These test materials were dissolved in 0.2 ml of 0.9% saline containing 0.1% bovine serum albumin and were intravenously injected through the intra-atrial cannula at the doses of 0.1, 1.0 and 10 μg/rat. Control rats were injected with only the vehicle. Blood samples (0.6 ml) were withdrawn into heparinized syringes from the same cannula before (-15 and 0 min) and 15, 30, 60 and 120 min after the injection of test materials. Blood samples were immediately cooled on ice and then centrifuged to obtain the plasma samples, which were stored at -20 °C until hormone extraction. Red cells were resuspended in physiologic saline and returned to the rats after each sampling.

To assess the pyrogenic activities of hIL-1β and its analogues, we administered these test materials or vehicle intraperitoneally to rats and measured the changes in rectal temperature using an electronic thermometer (model CTM-303; Terumo Co., Tokyo, Japan) every 2 hours for up to 8 hours after the injection.

Hormone assay

After extraction using silicic acid, plasma ACTH levels were determined by RIA using anti-ACTH rabbit antiserum (West), which was provided by the National Pituitary Agency of NIDDK, as described previously (5). The recovery ratio of the synthetic ACTH added to rat plasma by this extraction procedure was 88% and the results were not corrected by this value. Intra- and inter-assay coefficients of variation were both less than 10%, and the minimal detectable quantity of plasma ACTH by this assay system was 25 pg/ml.

Statistical analysis

Statistical analysis was performed by the analysis of variance and subsequent Bonferroni method; $P<0.05$ was considered to be significant. Results were expressed as the mean ± SEM of 5 rats in the experiments for the ACTH-releasing activities and of 3 rats in the experiments for pyrogenicities of the test materials.

RESULTS

Table 1 summarizes the pyrogenic activities of hIL-1β and its analogues in rats. At the doses of 0.1 to 10 μg/rat, hIL-1β elevated the rectal temperature in a dose-related manner 6 hours after the intraperitoneal injection, when the temperature usually reached its maximal level. Even at the highest dose tested, all of the analogues elevated the rectal temperature less than 1.0 °C. Under the same conditions, vehicle injection did not affect the rectal temperature.

Figure 1 compares the ACTH-releasing activities of hIL-1β and its analogues administered intravenously to conscious, freely- moving rats. At the dose of 1.0 μg/rats, all the test materials, except [Des-Ala^1, Asp^4]IL-1β, significantly elevated plasma ACTH levels. Under the same experimental conditions, vehicle injection did not alter plasma ACTH levels. The stimulatory effects of hIL-1β, [1-148]IL-1β, [7-153]IL-1β and [Des-Ala^1,

Table 1. Pyrogenic activities of hIL-1β and its analogues

Materials	Dose (μg/rat)		
	0.1	1.0	10
h IL-1β	±	+	++
[Gly^4] IL-1β	N.D.	N.D.	−
[Leu^{93}] IL-1β	N.D.	−	±
[7-153] IL-1β	N.D.	N.D.	−
[1-148] IL-1β	N.D.	N.D.	−
[Des-Ala^1, Asp^4] IL-1β	N.D.	N.D.	−

Pyrogenic activities were assessed by the changes of the rectal temperature (Δ°C) 6 hours after the intraperitoneal injection of the test materials in 3 rats of each group.
− : Δ°C<0.5 ± : 0.5≦Δ°C<1.0
+ : 1.0≦Δ°C<2.0 ++ : 2.0≦Δ°C<3.0
N.D. : not determined.

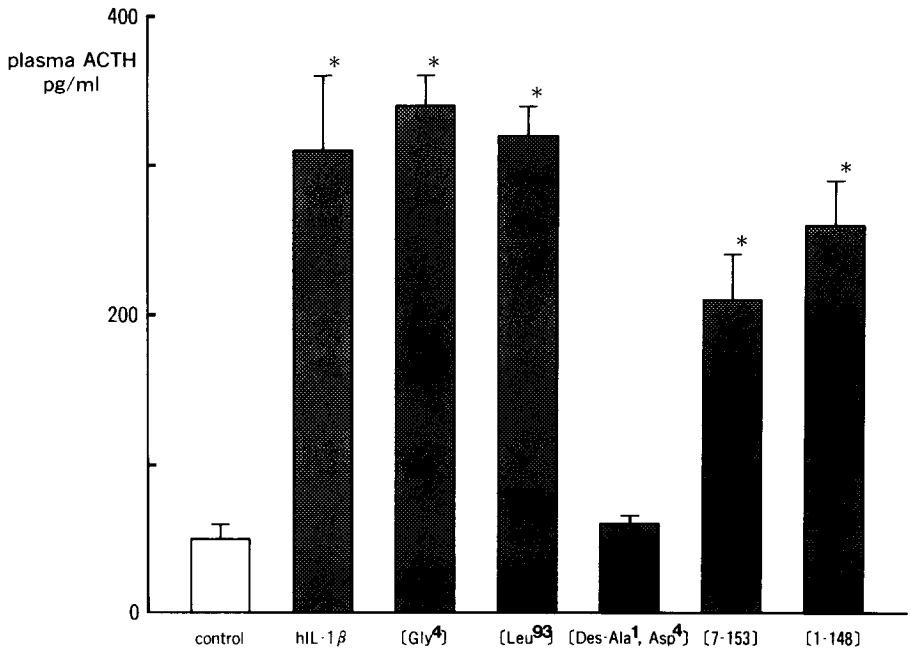

Figure 1. Maximal plasma ACTH levels after the intravenous administration of hIL-1β, [Gly^4]IL-1β, [Leu^{93}]IL-1β, [Des-Ala^1, Asp^4]IL-1β, [7-153]IL-1β and [1-148]IL-1β in conscious, freely-moving rats. Maximal values shown were usually observed 30 min after the intravenous administration of the test materials at the dose of 1.0 μg/rat. Under the same conditions, plasma ACTH levels of vehicle-injected control rats showed no significant changes from the basal level of 49.2 $\pm$ 6.6 (mean $\pm$ SEM, n=5) pg/ml. Each column represents the mean $\pm$ SEM of the maximal ACTH levels of 5 animals. *:$p<0.05$, compared to the vehicle-injected control rats.

Asp^4]IL-1β on plasma ACTH levels were further compared at different doses. As shown in figure 2, hIL-1β and [1-148]IL-1β elevated plasma ACTH levels dose-dependently between 0.1 and 10 μg/rat, and the effect was significant at the smallest dose tested (0.1 μg/rat). At all doses tested, the ACTH-releasing effect of [1-148]IL-1β was not significantly different from that of authentic hIL-1β. At the doses of 1.0 and 10 μg/rat, [7-153]IL-1β elevated plasma ACTH to significantly lower levels than those induced by hIL-1β and [1-148]IL-1β and, at a dose of 0.1 μg/rat, [7-153]IL-1β failed to stimulate ACTH secretion significantly. Since the dose-response curve of [7-153]IL-1β was parallel to those of hIL-1β in Figure 2, the ACTH-releasing activity of [7-153]IL-1β was calculated to be about 10 times less potent than that of authentic hIL-1β under these conditions. [Des-Ala^1, Asp^4]IL-1β, even at the largest dose tested (10 μg/rat), did not alter plasma ACTH levels throughout the experimental period.

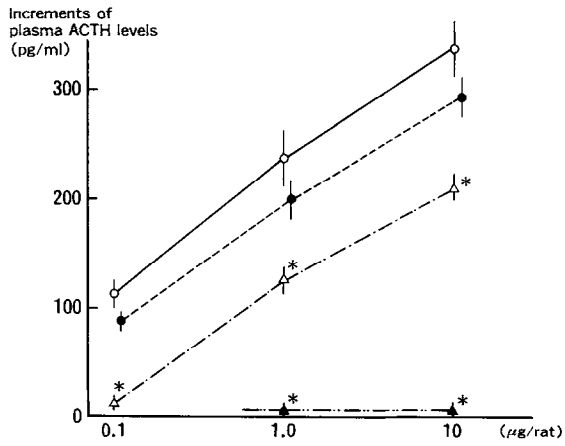

Figure 2. Dose-response effects of hIL-1β, [1-148]IL-1β, [7-153]IL-1β and [Des-Ala^1, Asp^4]IL-1β on the plasma ACTH levels. Maximal increases of the plasma ACTH concentrations were usually observed 30 min after the intravenous administration of the test material at all of the doses tested. Each point represents the mean ± SEM of the maximal increases in the plasma ACTH levels of 5 animals. (○):hIL-1β, (●):[1-148]IL-1β, (△)][7-153]IL-1β, (▲):[Des-Ala^1, Asp^4]IL-1β. *:p<0.05, compared to hIL-1β.

When the data shown in Figures 1 and 2 were assessed with the integrated amount of ACTH secreted during the sampling period, the conclusions drawn were the same as those described above (data not shown).

DISCUSSION

In the past few years, the structure-function relationships of the IL-1β molecule in the immunological and inflammatory activities of this polypeptide have been actively investigated, and the results suggest that various bioactivities of IL-1β may reside on different parts of the molecule (1,3,4). For studying the relationship between protein structure and function, modulation of the authentic molecule by site-specific mutagenesis is a powerful tool (7). In the present study, we demonstrated that some modifications of authentic hIL-1β resulted in a marked decrease in its pyrogenic activity with the preservation of considerable ACTH-releasing activity. These results suggest that the active domains of the authentic hIL-1β molecule responsible for the ACTH-releasing activity were different from those for pyrogenic activity.

Among the less-pyrogenic analogues tested, [Gly^4]IL-1β, [Leu^{93}]IL-1β and [1-148]IL-1β were demonstrated to have ACTH-releasing activity comparable to that of the authentic peptide. On the other hand, the removal of 6 amino

acids from the N-terminus resulted in about a 10-fold reduction in the ACTH-releasing activity, and removal of the alanine residue at the N-terminus combined with the substitution of an aspartic acid for the arginine residue at the 4th position made the peptide almost inactive. These results indicate that both the N-Terminal and C-terminal structures of hIL-1β are essential for the pyrogenic activity, and the N-terminal structure is more important than the C-terminal structure for its ACTH-secreting activity. It is not known why ACTH-releasing activity is much lower in [Des-Ala^1, Asp^4]IL-1β with the deletion of only one amino acid residue of the N-terminus and the substitution of the fourth amino acid residue rather than [7-153]IL-1β with the deletion of the first six amino acid residues of the N-terminus. Considering our previous findings that not Ala^1 but Arg^4 is critical to maintain the growth inhibitory factor (GIF) activity and receptor binding activity of hIL-1β molecule and, moreover, [Asp^4]IL-1β is much weaker than [Des-Arg^4]IL-1β in these activities, we suggest at present that the existence of Arg^4, probably as an alkaline amino acid, is also essential to maintain its ACTH-releasing activity.

Recently, the three dimensional structure of hIL-1β determined by X-ray crystallographic techniques disclosed that the N- and C-terminal residues of hIL-1β are close to each other and make the edge-like form of the tetrahedron-like structure of the whole molecule (8). The primary structure of the IL-1β receptor was also identified from the cloned complementary DNA isolated from the messenger-RNA of a mouse T cell line (9). However, it is still controversial whether the wide range of biological activities of IL-1β are exerted through a single class of receptor molecules or not, since competitive inhibition experiments using ^{125}I-labeled-hIL-1α or -hIL-1β suggested the existence of IL-1 binding molecules with heterogenous characteristics (10,11). Our present data clearly demonstrated that the two distinct activities of hIL-1β depend on different domains of this polypeptide and support the hypothesis that the diverse activities of IL-1β are not mediated through the interactions between a single type of receptor and the corresponding domain of the hIL-1β molecule. Further studies are necessary to fully elucidate the mechanisms of the IL-1-induced multiple biological activities.

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