# Effects of Recombinant Human Interleukins on Food Intake of Previously Food-Deprived Rats (43134)

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*Abstract.* **The effects of intracerebroventricular injection of recombinant human interleukin 1β (rhIL-1β), 1α (rhIL-1α), and 2 (rhIL-2) on feeding behavior were examined in previously food-deprived rats for 18 hr. At doses of 2–17 ng/rat, rhIL-1β significantly reduced food intake in a dose-dependent manner and the feeding suppression continued about 4 hr later. Only 17 ng/rat rhIL-1β reduced body weight gain for 8 hr after the injection. However, rhIL-1α at dose of 17 ng/rat did not show any significant change of food intake and body weight gain during the whole observation period. At both doses of 8 and 40 ng/rat, rhIL-2 also failed to suppress overfeeding after food deprivation. In adrenalectomized rats, feeding suppression by rhIL-1β appeared at the 1- to 2-hr time period. The present studies suggest that rhIL-1β may be, at least in part, involved in feeding suppression on various inflammatory processes and that adrenal hormones may not play an important role in the induction of feeding suppression by rhIL-1β.**

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Interleukins are cytokines and are synthesized by a wide variety of cells. Interleukin 1 (IL-1) mediates the acute phase reaction in the inflammatory responses (1) and IL-1 has been reported to have some direct effects on the central nervous system in eliciting the thermogenic response and hypersecretion of glucocorticoid and insulin (1–3). Moreover, a factor with IL-1β-like activity has been isolated from the brain (4) and IL-1β-immunoreactive fibers were found innervating the key endocrine and autonomic cell groups that control the central components of the acute reaction (5). These data suggest that IL-1 may be an important intrinsic neuromodulator in the brain. Therefore, it is supposed that interleukins also may be involved in a suppression of food intake in patients with severe infectious and inflammatory disease.

Some effects induced by central IL-1β administration appear to be mediated by the activation of the hypothalamus-pituitary-adrenal axis (1). Recently, Rothwell (3) demonstrated that the central effects by IL-1β on metabolic rate, body temperature, brown adipose tissue thermogenesis, and white cell counts of rats are mediated by release of corticotropin-releasing factor (CRF) (3). Various changes, elicited by central administration of IL-1β, may be related to the alterations of the hypothalamus-pituitary-adrenal axis, resulting in a increase of adrenal hormone release.

In the present studies, the effects of intracerebroventricularly injected recombinant human IL-1β (rhIL-1β), 1α (rhIL-1α), and 2 (rhIL-2) on feeding behavior were investigated in rats deprived of food for 18 hr. In addition, involvement of the hypothalamus-pituitary-adrenal axis in altered feeding behavior by the central administration of interleukins was also examined in adrenalectomized rats after starvation for 18 hr.

## Materials and Methods

**Animals.** The male Wistar rats were obtained from Imai animals laboratory (Saitama, Japan). The different groups of animals were used for all experiments and initial body weight of all rats was from 300 to 350 g in all experiment. The animals were individually housed in a temperature-controlled room (23 ± 1°C) with a 12-hr dark/12-hr light cycle (illumination from 6:00 to 18:00 hr). The animals were adapted to the powdered Purina laboratory chow (Oriental, Osaka, Japan) for at

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least 1 week before the cannula implantation and drinking water was given *ad libitum*. All experiments were initiated between 9:00 and 10:00 AM to eliminate the influence of diurnal variation in the responsiveness to chemicals. Food intake and body weight were measured to the nearest 0.1 g and drinking water was given *ad libitum*. access.

**Cannula Implantation.** Under pentobarbital anesthesia (40 mg/kg ip), a guide cannula of 23-gauge stainless steel tubing was stereotaxically implanted into the third ventricle 1 week before the infusion. The coordinate was chosen from the atlas of Konig and Klippel (6) and AP; +2.5 mm with respect to the bregma, midline, and D; −10.0 mm from the surface of the skull. The cannula was fixed to the skull with stainless screws and dental cement.

**Microinfusion of Chemicals.** A 29-gauge stainless steel tube connected through a long polyethylene tube to a 25-$\mu$l Hamilton microsyringe was inserted into the guide cannula. Chemicals were bolus infused into the third ventricle for a few minutes and the injection cannula was kept in the guide cannula for at least 3 min to block the reflux of chemicals. Following microinfusion, the animals were immediately returned to their own cages. Following the infusion, chronological food consumption and body weight gain for 2, 4, and 8 hr was measured.

**Experiment 1.** After food deprivation for 18 hr, each animal received intracerebroventricular infusion with rhIL-1$\beta$ (0.1, 0.5, 2, 8, or 17 ng/rat; Ohtsuka Pharmaceuticals, Co., Tokushima, Japan) or vehicle.

**Experiment 2.** After 18 hr of food deprivation, rhIL-1$\alpha$ (17 ng/rat; Ohtsuka Pharamecuticals, Co.) or vehicle was infused into the third ventricle in the same manner as in Experiment 1.

**Experiment 3.** After 18 hr of starvation, rhIL-2 (8 and 40 ng/rat; Takeda Pharmaceuticals, Co., Osaka, Japan) or vehicle was infused into the third ventricle in the same manner as in Experiment 1.

**Experiment 4.** All animals, in which the guide cannulas were previously implanted, were bilaterally adrenalectomized (ADX) through flank incision under light ether anesthesia. Following operation, 0.9% sodium chloride solution was available as drinking water. Seven days after adrenlectomy, rhIL-1$\beta$ (17 ng/rat) or vehicle was infused into the third ventricle after 18 hr of starvation. Residual adrenal tissues were anatomically checked on sacrifice, and serum corticosterone levels were also measured from blood samples collected by decapitation (7). Data from completely adrenalectomized animals were used for analysis.

**Preparation of Interleukins.** The biologic activities of interleukins are estimated by the mouse thymocyte [$^3$H]thymidine incorporation assay and, especially, lymphocyte-activating factor activity of rhIL-1$\beta$ is $2 \times 10^7$ half-maximal units/mg protein (8). The material has been judged to exhibit a purity of at least 99% based on analysis by high-performance liquid chromatography and polyacrylamide gel electrophoresis. The recombinant human interleukins have been found to contain the identical amino acid sequence predicted by the cDNA sequence.

**Statistical Analysis.** All data were expressed as mean ± SE. Statistical analysis of the means was performed by analysis of variance, followed by Duncan's multiple range test. When sizes of samples were unequal, the means of the group were used and the degree of freedom was reduced accordingly.

## Results

**Experiment 1.** Changes in food intake are presented in Figure 1. At a dose of 0.5 ng/rat, rhIL-1$\beta$ tended to decrease food intake at the 0- to 1-hr time period, but the difference was not statistically significant. The rhIL-1$\beta$ at a dose of 2 ng/rat significantly reduced food intake only for 1 hr after the injection. At both doses of 8 and 17 ng/rat, rhIL-1$\beta$ significantly suppressed food intake at the 0- to 1- and 1- to 2-hr periods. From 2 to 4 hr later, rhIL-1$\beta$ at a dose of 2–17 ng/rat tended to decrease food intake, but the differences were not statistically significant. Body weight gain for 8 hr after the injection was significantly reduced in rhIL-1$\beta$-treated animals only at a dose of 17 ng/rat

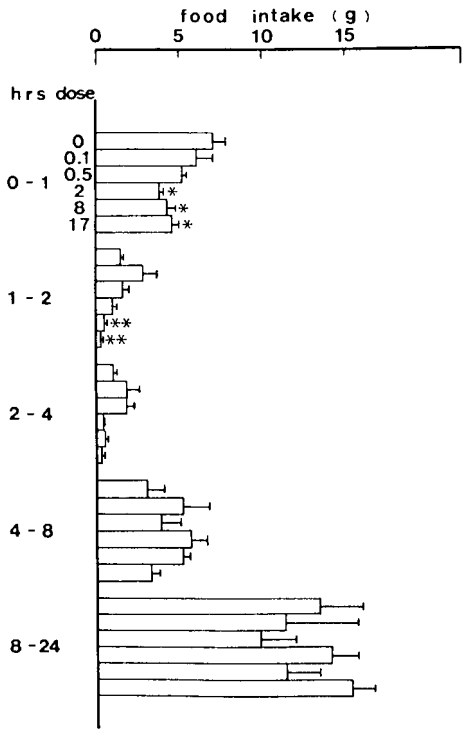


**Figure 1.** Changes of food intake after intracerebroventricular injection of rhIL-1$\beta$ at doses of 0 ($n$ = 5), 0.1 ($n$ = 6), 0.5 ($n$ = 5), 2 ($n$ = 7), 8 ($n$ = 6), and 17 ($n$ = 7) ng/rat in intact rats. * $P < 0.05$, ** $P < 0.01$ compared with the value of vehicle-treated animals.

(saline, 17.8 ± 4.3 g; IL-1$\beta$, 2 ng/rat, 19.5 ± 2.6 g, 8 ng/rat, 19.8 ± 3.6 g, 17 ng/rat, 8.3 ± 1.4 g ($P < 0.05$)).

**Experiment 2.** At a dose of 17 ng/rat, rhIL-1$\alpha$ did not show any significant change of food intake after food deprivation (Fig. 2). There was no change in body weight gain for 8 hr (vehicle, 17.0 ± 4.8 g, rhIL-1$\alpha$: 20.4 ± 2.3 g, not significant.

**Experiment 3.** At a dose of 8 ng/rat, rhIL-2 did not show any significant feeding suppression during the observation period for 24 hr (Fig. 3). Even at a higher dose (40 ng/rat), rhIL-2 failed to significantly inhibit food intake after food deprivation. There was no significant change in body weight for 8 hr among these groups (vehicle, 21.2 ± 2.9 g; rhIL-2, 40 ng/rat, 21.3 ± 3.5 g, not significant).

**Experiment 4.** In ADX rats, rhIL-1$\beta$ did not show a significant inhibition of food intake at the 0- to 1-hr time period (Fig. 4), whereas a significant decrease of food intake was observed at the 0- to 1-hr period in intact, same-aged rats at the same dose (17 ng/rat). (Fig. 1). From 1 to 2 hr later, a significant inhibition of feeding was observed in ADX rats. Feeding suppression tended to continue 4 hr later. However, body weight gain for 8 hr was not significantly changed in ADX rats (vehicle, −1.3 ± 4.4 g; rhIL-1$\beta$, 3.5 ± 2.1 g, not significant).

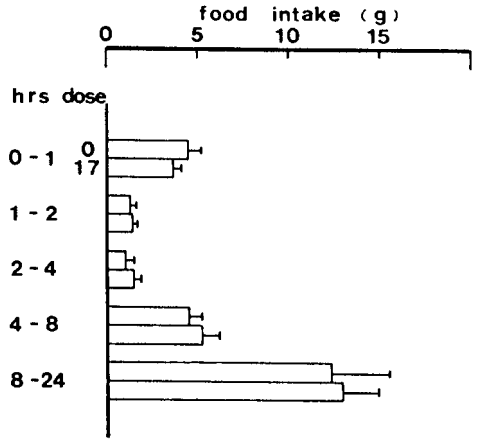


**Figure 2.** Changes of food intake after intracerebroventricular injection of rhIL-1$\alpha$ (17 ng/rat, $n = 6$) or vehicle ($n = 5$) in intact rats.

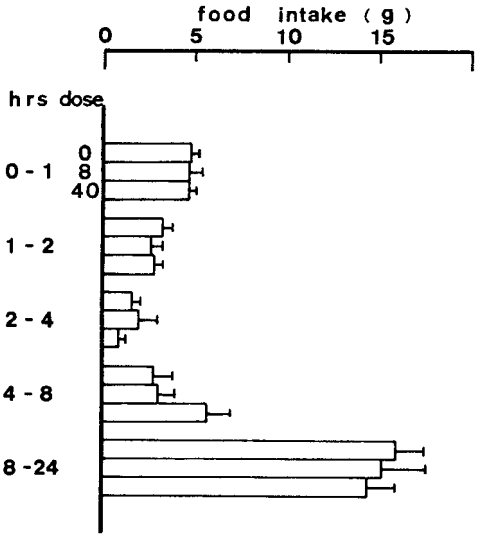


**Figure 3.** Changes of food intake after intracerebroventricular injection of rhIL-2 at doses of 0 ($n = 5$), 8 ($n = 6$), and 40 ($n = 8$) ng/rat in intact rats.

## Discussion

In this study, it was demonstrated that rhIL-1$\beta$ has a potent inhibition on food intake in rats deprived of food for 18 hr, accompanied by attenuation of body weight gain. Intracerebroventricular injection of rhIL-1$\beta$ suppressed food intake even at a dose of 2 ng/rat, while no significant change in food intake was observed with 17 ng/rat rhIL-1$\alpha$ administration at the same dose as the highest dose of rhIL-1$\beta$. Furthermore, rhIL-2 also failed to show a significant suppression of feeding even at a dose of 40 ng/rat, almost 20 times higher than rhIL-1$\beta$. Therefore, these results suggest that rhIL-1$\beta$ could be one of the possible candidates in cytokines that suppress food intake in severe inflammatory diseases.

In Experiment 1, suppression of food intake by rhIL-1$\beta$ was not dose related at the 0- to 1-hr time period. This can be explained by speculation that the dose of 2 ng/rat may be large enough to elicit a maximal response to the rhIL-1$\beta$ on the feeding behavior. In contrast, rhIL-1$\beta$ at a dose of 17 ng/rat had a greater effect on body weight than could be explained by the reductions in food intake over the period. Whereas there was only approximately a 2-g difference in food intake between the groups treated with 8 and 17 ng/rat rhIL-1$\beta$, the difference in body weight was about 11.5 g. This reduction in body weight gain cannot be ex-

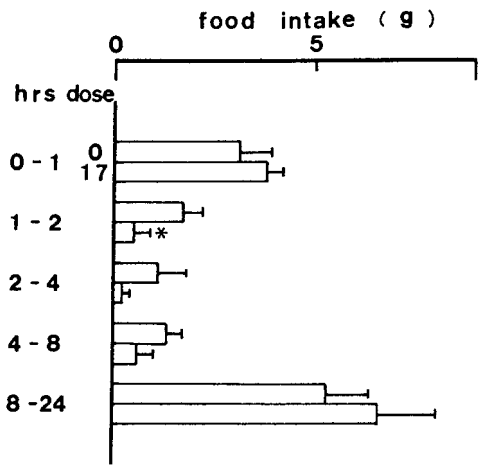


**Figure 4.** Changes of food intake after intracerebroventricular injection of rhIL-1$\beta$ (17 ng/rat, $n = 6$) or vehicle ($n = 7$) in adrenalectimized rats. * $P < 0.05$ compared with the value of vehicle-treated animals.

plained only by the suppressed food consumption. At a dose of 50 ng, intracerebroventricular injection of rhIL-1$\beta$ causes acute increases in colonic temperature, oxygen consumption, and brown adipose tissue activity in conscious rats (3). Intracerebroventricular administration of both 10 and 100 ng of rhIL-1$\beta$ is known to produce dose-related activation of the hypothalamus-pituitary-adrenal axis (9). In addition, injection of leukocyte supernatant containing IL-1 reduces gastric motility of goats to about 60% of controls (10). Therefore, it is possible that the modulation of thermogenesis or filling time of the digestive tract may affect the weight gain after the administration of rhIL-1$\beta$ and that there may be a difference between the responses on the feeding and those factors. However, further studies will be necessary to clarify the origin of this dissociation between the degree of decreased food intake and reduced weight gain.

Recently, Plata-Salaman *et al.* (11) have reported that intracerebroventricular infusion of rhIL-1$\beta$ suppressed food intake only during the dark cycle in *ad libitum*-fed rats, whereas it increased during the light cycle. They also demonstrated that a large dose of rhIL-1$\beta$ (13 ng/rat) reduced food consumption even 24 hr later. However, in the present study, even the highest dose of rhIL-1$\beta$ (17 ng/rat) showed its significant feeding inhibition only for 2 hr after the injection and at the beginning of the dark cycle. Nine hours after the injection, no suppression was observed at all with rhIL-1$\beta$ administration. This discrepancy can be explained by the difference of the experimental protocols. In our experiment, the infusion was performed in the light cycle after the animals were started for 18 hr. In contrast, previous investigators infused rhIL-1$\beta$ at the beginning of the dark cycle in *ad libitum*-fed animals. Furthermore, in our preliminary study, there was an obvious difference in the duration of feeding suppression by peripherally injected rhIL-1$\beta$ between *ad libitum*-fed and starved animals, since peripherally administered rhIL-1$\beta$ had a much longer duration (about 24 hr) in the feeding suppression. Therefore, the differences of infusion time and preloading starvation may affect the feeding responsiveness to centrally administered rhIL-1$\beta$.

Central administration of rhIL-1$\beta$ is known to stimulate CRF secretion from the paraventricular nucleus of the hypothalamus and result in an increase in circulating ACTH and glucocorticoid levels (3, 12). Uehara *et al.* (13) reported that the intravenous injection of rhIL-1$\beta$ significantly increased the plasma levels of adrenocorticotropic hormone in a dose-related manner, whereas rhIL-1$\alpha$ did not. A different spectrum of biologic activities in neuroendocrine actions is similar to our observations about the feeding response to interleukins. We have reported that microinjection of CRF into the lateral hypothalamic area (LHA), which is one of the important areas in the regulation of feeding behavior of rats (14), reduced the extracellular concentrations of norepinephrine and its metabolite in the lateral hypothalamus of food-deprived rats measured by an *in vivo* microdialysis method (15). Bilateral lesion of lateral hypothalamic area induces anorexia, resulting in the reduction of body weight gain with a decrease of sympathetic activity (16, 17). It is supposed that rhIL-1$\beta$ may suppress feeding through decreasing the neuronal activities in the LHA through stimulating hypothalamic CRF secretion. On the other hand, it was also demonstrated that electrophoretically applied rhIL-1$\beta$ directly inhibits glucose-sensitive neurons in the LHA (7). In Experiment 3, rhIL-1$\beta$ showed a delayed, but significant suppression of feeding in ADX rats, in which hypothalamic CRF concentration is supposed to be increased. Therefore, another possibility is that the observed suppression of food intake may be, at least in part, attributable to the direct inhibition of glucose-sensitive neuron firing in the LHA.

Withdrawal of corticosterone following adrenalectomy failed to abolish rhIL-1$\beta$-induced anorexia. In ADX rats, rhIL-1$\beta$-induced feeding inhibition was eliminated at the 0- to 1-hr time period and appeared at the 1- to 2-hr period. Because it has been shown that corticosteroid blocks interleukin 1 transcription and reduces its production (18), it is possible that withdrawal of corticosterone may increase the local concentration of rhIL-1$\beta$. The supposed down-regulation of brain rhIL-1$\beta$ receptors may contribute to the delayed response to the exogenously injected rhIL-1$\beta$. In addition, McCarthy *et al.* (19) pointed out the possibility that rhIL-1$\beta$-induced anorexia seems to be mediated by the altered liver metabolism. It might be speculated that alteration in liver metabolism following withdrawal of adrenal hormones may participate in the delayed appearance of rhIL-1$\beta$-induced feeding inhibition.

The present studies suggested that rhIL-1$\beta$ may be one of the important cytokines in suppressing food intake on various inflammatory processes. Existence of adrenal hormones may not play an important role in the induction of anorexia by rhIL-1$\beta$, since the degree of rhIL-1$\beta$-induced feeding inhibition was not influenced by withdrawal of adrenal steroids after adrenalectomy.

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