These experiments show that current x-ray lasers are sufficiently well-characterized and have enough brightness to be used successfully in the creation of x-ray holograms. In addition x-ray mirrors now have the necessary flatness, roughness, and reflectivities to allow their use in phase-sensitive x-ray experiments. These experiments also show that with x-ray lasers, holograms can be created with exposure times of a few hundred picoseconds. The trend in x-ray laser development is toward decreasing wavelengths and improved coherence. Lasing has been observed down as low as 6.6 nm in nickel-like europium and 5.03 nm in nickel-like ytterbium (*18*). This scheme should be scalable to wavelengths below 4.4 nm in nickel-like tungsten. There have been proposals for improving the spatial coherence of x-ray lasers (*19*). X-ray laser cavities with partially transmitting x-ray beam splitters as output couplers have also been demonstrated (*20*). This offers the possibility of both improved efficiency and coherence. With laser wavelengths in the water window (4.4 to 2.3 nm) where there is high contrast between protein and water, x-ray holograms of living cells can be produced.

REFERENCES AND NOTES

1. S. Aoke and S. Kikuta, *Jpn. J. Appl. Phys.* **11**, 1857 (1972).
2. M. Howells *et al.*, *Science* **238**, 514 (1987).
3. D. Gabor, *Proc. R. Soc. London Ser. A* **197**, 454 (1949).
4. T. Barbee, *Opt. Eng.* **25**, 898 (1986).
5. D. Matthews *et al.*, *Phys. Rev. Lett.* **54**, 110 (1985).
6. P. Hagelstein *et al.*, *Phys. Rev. A* **34**, 1931 (1986).
7. D. Whelan, *et al.*, in preparation.
8. A. Siegman, *Lasers* (University Science Books, Mill Valley, CA, 1986).
9. D. Matthews *et al.*, *J. Opt. Soc. B* **4**, 575 (1987).
10. The mirror substrate was fabricated by General Optics, Moorpark, CA 93021.
11. The surface measurements were performed by N. Brown and H. Bissinger of the LLNL Optics Shop. See also N. J. Brown, *Soc. Photo-Optical Instrum. Eng.* **680**, 141 (1986).
12. D. Stearns *et al.*, *ibid.* **688**, 91 (1986).
13. B. Henke *et al.*, *J. Opt. Soc. B*, **1**, 818 (1984); B. Henke *et al.*, *ibid.*, p. 828.
14. The visible and ultraviolet wavelength blocking filter was made by Luxel Corporation, Friday Harbor, WA 98250.
15. M. Born and E. Wolf, *Principles of Optics* (Pergamon, New York, ed. 3, 1986), p. 510.
16. A. Baez, *J. Opt. Soc.* **42**, 756 (1952).
17. H. Fujiwara and K. Murata, *Opt. Acta* **19**, 85 (1972).
18. B. MacGowan *et al.*, in preparation.
19. M. Rosen, J. Trebes, D. Matthews, *Comm. Plasma Phys. Controlled Fusion* **10**, 245 (1987).
20. N. Ceglio *et al.*, in preparation.

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Release of Multiple Hormones by a Direct Action of Interleukin-1 on Pituitary Cells

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Exposure to bacterial endotoxins has long been known to stimulate the release of anterior pituitary hormones; administration of endotoxin was at one time a common clinical test of anterior pituitary function. Endotoxin is a potent stimulus for production of the endogenous pyrogenic protein, interleukin-1 (IL-1), by macrophages and monocytes. The possibility that IL-1 has a direct effect on the secretion of hormones by rat pituitary cells in a monolayer culture was investigated. Recombinant human IL-1β stimulated the secretion of adrenocorticotropic hormone, luteinizing hormone, growth hormone, and thyroid-stimulating hormone. Increased hormone secretion into culture supernatants was found with IL-1 concentrations ranging from $10^{-9}M$ to $10^{-12}M$. Prolactin secretion by the monolayers was inhibited by similar doses. These concentrations of IL-1 are within the range reported for IL-1 in serum, suggesting that IL-1 generated peripherally by mononuclear immune cells may act directly on anterior pituitary cells to modulate hormone secretion in vivo. Incubation of IL-1 solutions with antibody to IL-1 neutralized these actions. These pituitary effects of IL-1 suggest that this monokine may be an important regulator of the metabolic adaptations to infectious stressors.

INTERLEUKIN-1 (IL-1), A MONOKINE secreted predominantly by stimulated macrophages and monocytes, increases the proliferation of antigen-stimulated lymphocytes and the production of lymphokines (*1*). In addition to these well-described effects on immune function, IL-1 also suppresses adipocyte lipoprotein lipase activity in vitro (*2*), stimulates hepatic synthesis of fibrinogen and iron-binding proteins (*1*), downregulates hepatocyte glucocorticoid receptors and gluconeogenesis (*3*), and increases prostaglandin production by various cells, including fibroblasts, chondrocytes, and endothelial cells (*1, 4*). Other hormone-like regulatory effects of IL-1 are demonstrated by its actions on the classical endocrine systems. For example, IL-1 inhibits the release of insulin from islet cells in vitro (*5*) and stimulates the secretion of adrenocorticotropic hormone (ACTH) and cortisol when it is injected into mice (*6*). IL-1 and another monokine, hepatocyte-stimulating factor, have been reported to stimulate ACTH secretion by AtT-20 cells, a mouse pituitary tumor line (*7*).

Although these studies showed that IL-1 has an effect on specific hormones of the pituitary-adrenal axis, the possible effects of IL-1 on other anterior pituitary hormones are unknown. Also unknown is whether the pituitary responses to IL-1 are mediated by neuroendocrine actions on the hypothalamus or are a consequence of direct actions on pituitary cells. To address these questions, we examined the effects of recombinant human and mouse IL-1 on secretion of ACTH, luteinizing hormone (LH), prolactin (PRL), growth hormone (GH), and thyroid-stimulating hormone (TSH) by monolayers of rat anterior pituitary cells in short-term culture.

Using 3-day cultures of cells obtained from pituitaries of normal female Sprague-Dawley rats (*8*), we found that recombinant murine (*9*) and human IL-1 (*10*) stimulated the release of ACTH, LH, GH, and TSH. This stimulation was dose-dependent (Fig. 1). Murine IL-1 also increased the secretion of all four hormones at concentrations of $10^{-8}M$ to $10^{-10}M$, but this effect was less clearly related to dose. Prolactin secretion, in contrast, was inhibited by both murine and human IL-1 in doses of $10^{-9}M$ to $10^{-12}M$ in various experiments (Fig. 2).

Since the recombinant IL-1 preparations we used were derived from Gram-negative bacteria, it was important to establish whether these endocrine actions of our IL-1 could be a consequence of endotoxin contamination. Heat denaturing (*11*) completely abolished the ability of human IL-1 to alter hormone secretion from pituitary monolayers. Since this treatment would not inactivate any endotoxin contaminating the recombinant preparation, these heat-denaturing experiments demonstrate that endotoxin contaminants cannot be mediating the hormonal effects observed in these studies. Moreover, when human IL-1 was preincubated with specific antibody to recombinant

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Table 1. Effects of incubation of rat anterior pituitary cells with $10^{-10}M$ IL-1 with or without antibody to human IL-1 (*12*). Values are means ± SEM for four to six determinations in each experiment.

ACTH (pg/ml)	GH (ng/ml)	LH (ng/ml)	TSH (ng/ml)
	Without antibody		
2967 ± 344	7163 ± 999	14.1 ± 1.6	4.5 ± 0.6
	With antibody		
947 ± 99*	2827 ± 275**	3.5 ± 0.6**	1.5 ± 0.2*

*$P < 0.05$, **$P < 0.01$ by nonpaired Student's t test.

human IL-1 (*12*) all of the effects of IL-1 on pituitary monolayers were significantly neutralized (Table 1).

We found that IL-1 acts directly on pituitary cells to stimulate the release of ACTH, LH, TSH, and GH while simultaneously inhibiting the release of PRL. These effects occurred at concentrations of IL-1 similar to those observed in vivo. In normal volunteers and in patients with sepsis, IL-1 activity equivalent to that of $1 \times 10^{-10}M$ to $2 \times 10^{-9}M$ was demonstrated in plasma (*13*). Our studies also suggest that the stimulation of ACTH release in vivo following IL-1 injection reported by Besedovsky *et al.* (*6*) may have been mediated by direct pituitary actions of IL-1. In their studies, the injection of 1000 ng of IL-1 probably resulted in circulating levels exceeding the concentration of 17 ng/ml ($10^{-9}M$) that elicited maximal ACTH secretion in our pituitary monolayer studies.

IL-1 has been shown to stimulate prostaglandin E_2 (PGE_2) in fibroblasts and other cell lines (*4*). We observed that mouse IL-1 also increased PGE_2 secretion by pituitary monolayers (29.8 ± 8 pg/ml in media with $10^{-10}M$ IL-1 versus 3.9 ± 0.8 in controls). However, the stimulatory effects of IL-1 on hormone secretion do not appear to be mediated by PGE_2, since addition of indomethacin at 3 or 6 μg/ml blocked IL-1–stimulated PGE_2 secretion without affecting the stimulation of ACTH or LH release. Arachidonate metabolites may mediate other actions of IL-1, such as induction of IL-2 secretion by T cells, but these appear to be products of the lipoxygenase, not the prostaglandin synthetase pathway (*14*).

The absence of capillary perfusion exposes cells in monolayer cultures to high concentrations of secretory products that accumulate in the media. Since ACTH release by clonal corticotrophs is directly enhanced by IL-1 (*7*), we considered the possibility that the effects of IL-1 observed on other pituitary hormones were due to the direct actions of ACTH. Therefore, pituitary cells were incubated with ACTH at concentrations comparable to those measured in wells containing IL-1–stimulated monolayers. These concentrations (2000 and 5000 ng/ml) did not increase secretion of LH, TSH, or GH, thereby excluding a paracrine effect of ACTH on the secretion of these hormones. This finding is consistent with the results of another study in which, under conditions similar to ours, no interactions of multiple releasing factors or anterior pituitary hormones on pituitary cells in monolayer culture were found (*15*). Moreover, in preliminary studies with perifused pituitary fragments, we noted that both mouse and human IL-1, at concentrations similar to those added to pituitary monolayers, stimulated a rapid release of GH, ACTH, TSH, and LH (*16*). Since perifusion greatly reduces accumulation of secretory products in the media, this provides further evidence that hormones or cytokines elaborated by the pituitary do not mediate any of the stimulatory responses to IL-1. Although our data show that IL-1 is a potent pituitary secretagogue, further studies will be required to determine its precise subcellular loci of action.

In contrast to the result of an earlier study in which corticotropin-releasing factor was used (*15*), incubation of pituitary monolayers with ACTH inhibited PRL secretion by 23%. Furthermore, the maximal inhibition of PRL occurred at the same doses of IL-1 as the maximal stimulation of ACTH. From these data, it is possible that the inhibition of PRL secretion by IL-1 added to this monolayer system is mediated indirectly through its stimulatory effect on ACTH secretion.

Brodish (*17*) reported that "tissue corticotropin-releasing factors" appeared in the

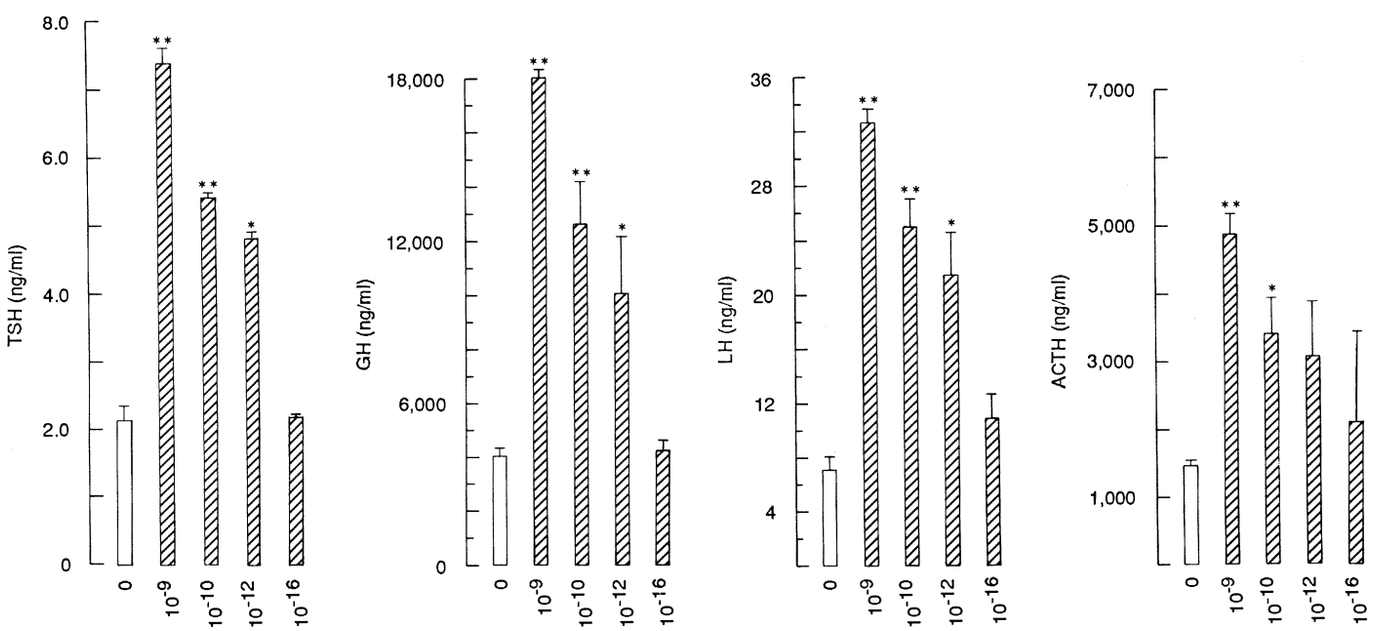

Fig. 1. Effects of recombinant human IL-1 ($10^{-9}M$ to $10^{-12}M$) on secretion by rat anterior pituitary cells in vitro. Details of techniques are in (*8*). IL-1 (hatched bars) significantly stimulated TSH, GH, LH, and ACTH secretion (mean ± SE) over control wells (open bars). Statistical analyses were performed by nonpaired two-tailed Student's t test with a Bonferroni correction for four dependent measures per variable. *$P < 0.05$; **$P < 0.005$.

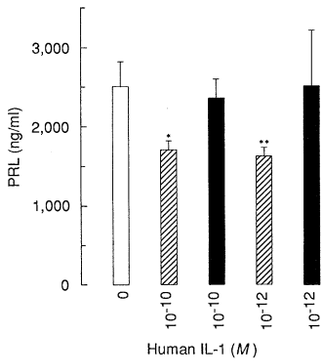

Fig. 2. Effects of incubation of rat anterior pituitary cells with $10^{-10}M$ human IL-1 with or without antibody to human IL-1 (*12*). Control levels (mean ± SE) are shown by the open bar, IL-1 alone by hatched bars, and incubation with IL-1 and antibody by solid bars. $*P < 0.05$, $**P < 0.005$ by nonpaired two-tailed Student's t test with Bonferroni correction.

blood of rats after abdominal surgical stress, resulting in activation of the pituitary-adrenal axis. Our data and those of others (*6, 7*) suggest that this factor may well be IL-1, released after inflammatory responses are initiated. Although IL-1 appears to act directly on pituitary cells, it is also possible that IL-1 may act indirectly on pituitary secretion through hypothalamic factors. The validation of a pituitary site of endocrine actions does not rule out additional suprapituitary actions of IL-1 via effects on other neuroendocrine systems. For example, brain astrocytes were reported to produce IL-1 (*18*), and IL-1 was extracted from the brains of endotoxin-treated mice (*19*). IL-1 also increased PGE_2 release in the hypothalamus, a probable pyrogenic mechanism (*20*), and was reported to displace opiate ligands bound to brain sections (*21*). Such data suggest that IL-1 could modulate, by these or other mechanisms, aminergic or peptidergic neurotransmitters affecting anterior pituitary hormone release or other central nervous system responses.

Our data show that the processes of infection or inflammation and consequent immune activation may serve as potent, direct pituitary stimuli for the secretion of ACTH, LH, TSH, and GH while inhibiting PRL. We, and others, have observed that PRL is an important physiologic immunopermissive hormone in vivo (*22*). In contrast, ACTH release results in elevations of endogenous adrenal corticosteroids, which have immunosuppressant and anti-inflammatory effects. The combined effects of increased IL-1 on raising corticosteroid levels and decreasing PRL may thus serve as a mechanism to limit the acute physiological responses otherwise initiated by increased systemic levels of IL-1. With chronic infection or inflammation, sustained IL-1 levels may ultimately deplete pituitary hormones, resulting in their decreased concentration. Finally, IL-1 may act in concert with more classically defined stress-responsive neuroendocrine mechanisms to further modulate the secretion of pituitary hormones in response to systemic infections.

Definition of important physiological networks between the neuroendocrine and immune systems has only recently begun. For example, as shown herein, many of the anterior pituitary hormones may be shared messengers under the control of both the immune and nervous systems. ACTH, endorphins, PRL, and TSH apparently bind to lymphocytes and can modulate lymphocyte function (*22, 23*). Furthermore, lymphocytes synthesize and secrete ACTH, endorphins, TSH, and gonadotropins in response to various immunologic stimuli (*24*). The sex steroids under control of pituitary gonadotropins also have immunoregulatory effects (*25*). The results of the present studies illustrate a direct mechanism by which an important immunologic messenger (IL-1) can affect neuroendocrine function, an example of the dynamic regulatory interactions between the immune and neuroendocrine systems.

REFERENCES AND NOTES

1. C. A. Dinarello, *Rev. Infect. Dis.* **6**, 51 (1984).
2. B. A. Beutler and A. Cerami, *J. Immunol.* **135**, 3969 (1985).
3. M. Hill, R. Stith, R. McCallum, *ibid.* **137**, 858 (1986).
4. C. R. Albrightson, N. L. Baenziger, P. Needleman, *ibid.* **135**, 1872 (1985).
5. K. Bendtzen *et al.*, *Science* **232**, 1545 (1986).
6. H. Besedovsky, A. del Rey, E. Sorkin, C. A. Dinarello, *ibid.* **233**, 652 (1986).
7. B. M. R. N. J. Woloski, E. M. Smith, W. J. Meyer III, G. M. Fuller, J. E. Blalock, *ibid.* **230**, 1035 (1985).
8. Female Sprague-Dawley rats (180 to 250 g) at random stages of the estrous cycle were housed for 10 to 60 days at two to three animals per cage under 12 hours of light daily. After they were killed by decapitation, anterior pituitaries were removed and washed three times with Dulbecco's modified Eagle's medium (DMEM) with 25 mM Hepes buffer, 2 mM L-glutamine, D-glucose (1000 mg/liter), sodium pyruvate (110 mg/liter) plus an antibiotic-antimycotic mixture (ABM) (all from Gibco). Twenty to forty pituitaries were minced into 1- to 2-mm diameter fragments, pooled, washed with Hanks calcium- and magnesium-free (CMF) medium (Gibco), and enzymatically dispersed by incubation at 37°C in 0.4% collagenase (CLS IV; Cooper Biomedical) for 30 minutes and then with deoxyribonuclease I (200 μg/ml) (Sigma) for 2 minutes. The pituitary cells were then centrifuged at 400*g*, and the pellet was washed three times, mechanically dispersed, and centrifuged through a mixture of 4% bovine serum albumin and Hanks CMF, which removed >95% of the remaining red blood cells. The pellet was resuspended to 300,000 cells per milliliter in DMEM with 10% fetal bovine serum (FBS) (Gibco lot 27K1055, heat-inactivated) and ABM and plated into 24-well culture plates at 1 ml per well. The FBS contained estradiol (15.07 pg/ml), which yielded 5.5 pM estradiol in the culture medium. After a 3-day incubation, cells in monolayers were washed and incubated with serum-free medium (DMEM with 0.1% bovine albumin) for 4 hours. This was then removed, and sextuplicate control wells were filled either with 1 ml of this medium or $10^{-9}M$ heat-denatured IL-1. Other wells were filled in quadruplicate with medium containing either thyrotropin-releasing hormone (TRH), corticotropin-releasing factor, growth hormone–releasing factor (GHRF), or luteinizing hormone releasing hormone (LHRH) (all from Sigma) or with various concentrations of recombinant murine or human IL-1. After 4 hours, medium was aspirated from each well, and concentrations of PRL, LH, ACTH, GH, and TSH were determined by radioimmunoassay (reagents supplied by the National Hormone and Pituitary Program). The trophic hormones were stimulated only by their specific releasing factor. In representative experiments, LHRH ($10^{-8}M$) stimulated LH by 1040%; TRH ($10^{-6}M$) increased TRH by 205% and PRL by 150%; CRF ($10^{-8}M$) raised ACTH levels by 1030%; and GHRF ($10^{-8}M$) induced a 414% rise in GH. At least one of these positive controls was included in each study to test cellular responsiveness. The addition of IL-1 ($10^{-9}M$) to control media resulted in no detectable levels of the trophic hormones in the radioimmunoassays.
9. Mouse recombinant IL-1α with specific activity of 6×10^6 thymocyte units per milligram was kindly supplied by P. Lomedico, Hoffmann–La Roche, Nutley, NJ.
10. Human recombinant IL-1β was purchased from Cistron Technology, Pinebrook, NJ. The specific activity was 1×10^6 thymocyte units per milligram.
11. Human IL-1 was denatured by heating to 98°C for 1 hour.
12. Polyclonal rabbit antibody to human IL-1 was incubated with human IL-1 at a serum dilution of 1:1250 for 6 hours. At this dilution, neither antibody alone nor normal rabbit serum affected hormone secretion by pituitary monolayers.
13. C. A. Dinarello, G. H. A. Clowes, A. H. Gordon, C. A. Saravis, S. M. Wolff, *J. Immunol.* **133**, 1332 (1984); A. Luger, H. Graf, H. P. Schwarz, H. K. Stummvoll, T. A. Luger, *Crit. Care Med.* **14**, 458 (1986).
14. W. L. Farrar and J. L. Humes, *J. Immunol.* **135**, 1153 (1985).
15. W. Wehrenberg *et al.*, *Endocrinology* **114**, 1995 (1984).
16. E. W. Bernton *et al.*, unpublished data.
17. A. Brodish, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36**, 2088 (1977).
18. A. Fontana and P. Grob, *Lymphokine Res.* **3**, 11 (1984).
19. A. Fontana, E. Weber, J. Dayer, *J. Immunol.* **133**, 1696 (1984).
20. C. Dinarello and H. Bernheim, *J. Neurochem.* **37**, 702 (1981).
21. M. Ahmed, J. Llanos, C. Dinarello, C. Blatteis, *Peptides* **6**, 1149 (1985).
22. E. Bernton, D. Hartmann, M. Gilbreath, J. Holaday, M. Meltzer, in *Leukocytes and Host Defense*, J. Oppenheim, Ed. (Liss, New York, 1986); P. C. Heistand, P. Mekler, R. Nordmann, A. Greider, C. Permmongkol, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 2599 (1986); E. Nagy, I. Berczi, G. E. Wren, S. L. Asa, K. Kovacs, *Immunopharmacology* **6**, 231 (1986); D. M. Larson, *Prog. Allergy* **38**, 222 (1986).
23. C. J. Heijnen, C. Bevers, A. Kavelaars, R. E. Ballieux, *J. Immunol.* **136**, 213 (1986); T. E. Kruger and J. E. Blalock, *ibid.* **137**, 197 (1986); R. N. Mandler, W. E. Biddison, R. Mandler, S. Serrate, *ibid.* **136**, 934 (1986); H. M. Johnson, B. A. Torres, E. M. Smith, L. D. Dion, J. E. Blalock, *ibid.* **132**, 246 (1984).
24. E. M. Smith and J. E. Blalock, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7530 (1981); E. M. Smith, M. Phan, T. E. Kruger, D. H. Coppenhaver, J. E. Blalock, *ibid.* **80**, 6010 (1983); D. Harbour-McMenamin, E. M. Smith, J. E. Blalock, *ibid.* **83**, 6834 (1986).
25. O. Pung, A. Tucker, A. Vore, M. Luster, *Infect. Immun.* **50**, 91 (1985); M. Luster *et al.*, *J. Immunol.* **133**, 110 (1984).

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