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HUMAN LEUKOCYTE INTERFERON (HuIFN-α): POTENT ENDORPHIN-LIKE OPIOID ACTIVITY

J. Edwin Blalock and Eric M. Smith

Department of Microbiology, University of Texas Medical Branch
Galveston, Texas 77550

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SUMMARY: Human leukocyte interferon, but not fibroblast or immune interferons, binds to opiate receptors in vitro. When injected intracerebrally into mice, human leukocyte, but not fibroblast or immune interferon, caused potent endorphin-like opioid effects. These effects include analgesia, lack of spontaneous locomotion and catalepsy. All of these actions of human leukocyte interferon were preventable and reversible by the opiate antagonist naloxone. The findings suggest that some of the side effects of leukocyte interferon therapy may be mediated by opiate receptor binding. They also provide evidence for a regulatory circuit between the immune and neuroendocrine system. This putative circuit could be an etiologic site for certain psychopathological states.

INTRODUCTION

We have recently shown that human lymphocytes stimulated with HuIFN-α inducers produce ACTH and endorphin-like substances that are apparently intrinsically associated with HuIFN-α (1).* These studies suggest a regulatory circuit between the immune and neuroendocrine systems which may involve known neuroendocrine hormones, some of which are HuIFN-α associated. To test the possibility that a product of the immune system (HuIFN-α) that is structurally related to neuroendocrine hormones (ACTH and endorphins) might affect the neuroendocrine system, we have studied the possible endorphin-like opioid activity of HuIFN-α. We report here the binding of HuIFN-α to opiate receptors in vitro and its potent endorphin-like analgesic and tranquilizing activity in vivo. These findings support the existence of a regulatory circuit between the immune and neuroendocrine systems, as well as provide a possibly new etiologic

*IFN=interferon; HuIFN=human interferon; HuIFN-α=human leukocyte interferon; HuIFN-β=human fibroblast interferon; HuIFN-γ=human immune interferon

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site for psychopathological states. Practically, they may be of immediate concern in terms of the observed side effects of HuIFN-α therapy of human diseases.

MATERIALS AND METHODS

Human interferons (HuIFNs) were prepared as previously described (1,2). HuIFN-α was purified to 10^6 U/mg protein by the method of Mogensen and Cantell (3). Purification to $10^{8.3}$ U/mg protein was by affinity chromatography on an anti-ACTH-α (1-13) antibody sepharose column. Homogeneous HuIFN-α is considered to have a specific activity of $10^{8.3}$ to $10^{8.5}$ U/mg protein (4).

HuIFNs, β-endorphin (Bio-Ria, Brussel, Belgium) and morphine sulfate (Elkins-Sinn, Inc., Cherry Hill, NJ) were tested for the ability to bind to opiate receptors as measured by inhibition of specific [^{3}H]dihydromorphine binding to mouse brain tissue (5). The particulate fraction of homogenized ICR mouse brains was resuspended at 2% (w/v) in 2 ml of 50 mM Tris, HCl buffer, pH 7.4 and incubated for 5 min with 0.1 ml of the sample (at the described concentration) at 37°C. Next 0.1 ml of [N-Methyl ^{3}H]-dihydromorphine (72 Ci mmol^{-1}, 4 x 10^{-9} M) was incubated with the brain fraction for 15 min at 37°C. The brain mixture with bound [^{3}H]dihydromorphine was collected on a glass fiber filter (type A/E, Gelman Instrument Co.; Ann Arbor, MI), washed with cold Tris-HCl buffer, and the bound isotope quantitated by standard liquid scintillation techniques. Incubations were carried out in duplicate and specific binding was defined as that fraction of the bound [^{3}H]dihydromorphine displaced by 10^{-4} M naloxone. In each assay, approximately 6,000 cpm of [^{3}H]dihydromorphine bound specifically and 3,000 cpm bound nonspecifically.

The above substances were assayed for biologic activity by intracerebral injection of 50 µl into male ICR mice (6). The mice were tested for analgesia by a hot plate test (7) conducted at 50±0.5°C at 5 min post inoculation. When the mice began vigorous licking of their paws, the test was stopped and the time interval on the hot plate recorded. The percent analgesia was calculated according to Tseng et al. (8) as $[(T_1-T_0)/(T_2-T_0)] \times 100$. The control latency ($T_0$) was the mean response time of mice injected intracerebrally with diluent. This time did not differ from noninjected controls. Test latencies (T_1) were measured at 5 min post-injection for each mouse. The cutoff time (T_2) of the hot plate test was 60 sec. Immobility was defined as a lack of spontaneous movement and graded on a scale of (-) to (4+). (4+) was the absence of spontaneous movement and (-) indicates complete spontaneous mobility. Four to six mice were used for each concentration of each sample.

RESULTS

Human interferons comprise at least three antigenically and structurally distinct proteins termed leukocyte interferon (HuIFN-α), fibroblast interferon (HuIFN-β) and immune interferon (HuIFN-γ) (for review see 9). These HuIFNs were prepared as previously described (1,2) and tested for their ability to reduce the specific binding of [^{3}H]dihydromorphine to a membrane preparation

from mouse brain (5). HuIFN-α, but not HuIFN-β or γ, inhibited the specific binding of [^{3}H]dihydromorphine (Table 1). This finding is consistent with the antigenic relatedness of HuIFN-α, but not HuIFN-β or γ, to endorphin (1). Though there was an initial 10 fold loss of activity with purification, opiate receptor binding activity was still evident with apparently homogeneous HuIFN-α (4). Thus the binding activity appears to be an integral part of the HuIFN-α molecule. The above described loss in activity seems to be due to smaller molecular weight opiate receptor binding substances which do not co-purify with HuIFN-α. When crude and partially purified HuIFN-α was subjected to SDS-urea polyacrylamide gel electrophoresis, opiate receptor binding activity was detected with HuIFN-α as well as in several low molecular weight fractions. These low molecular weight forms are currently under study. Comparing the doses required for 50% inhibition (I_{50}) of specific binding shows that HuIFN-α ($10^{8.3}$ U/mg protein) [I_{50}, 5000 U/ml, about 10^{-9} M] is about 320 times more active on a molar basis than morphine (I_{50}, about 3.2×10^{-7} M). The molarity and molar amounts of HuIFN-α are calculated on the basis of a specific activity of $10^{8.3}$ U/mg protein and an average molecular weight of 20,000 daltons.

To determine if the opiate receptor binding (or lack of binding) corresponded to opiate activity *in vivo*, the different HuIFNs were injected intracerebrally in a volume of 50 µl into male ICR mice weighing 25-30g (Timco Laboratories) (6). Other animals were injected similarly with β-endorphin, morphine or balanced salt solution. After injections, mice were observed for gross abnormalities of behavior and tested for analgesia by a hot plate test (7). As with opiate receptor binding, only HuIFN-α (not β or γ) showed analgesic activity. This effect was observed with crude as well as apparently homogeneous HuIFN-α and occurred at concentrations similar to those that showed opiate receptor binding (Table 1). Based on a rough calculation, HuIFN-α (5000 U, 10^{-3} nmole) was about 8000 and 1000 times more active than morphine (8.6 nmole) and β-endorphins (1.4 nmole M), respectively. Thus HuIFN-α is a very potent analgesic compound. Naloxone (1 mg/kg) given intraperitoneally

Table 1. Endorphin-like opioid activity of HuIFN-α

Sample		% Inhibition of Specific ^{3}H-dihydromorphine Binding	Analgesic Activity Response Time in sec + S.D. (% Analgesia) No Naloxone	Naloxone Reversal	Naloxone Pretreatment	Immobility
HuIFN-β (10^4 U/mg protein)	500 U	0	22.5 ± 1.7 (0.2)	ND	ND	-
HuIFN-γ (10^5 U/mg protein)	250 U	1.3	24.3 ± 2.1 (5.0)	ND	ND	-
HuIFN-α (10^4 U/mg protein)	500 U	45.4	59.0 ± 3.3 (97.3)	22.1 ± 6.0 (0)	23.7 ± 1.2 (3.4)	4+
	250 U	30.2	45.3 ± 12.7 (60.9)	ND	ND	2+
(10^6 U/mg protein)	7500 U	65.0	60.0 ± 0 (100)	21.8 ± 6.2 (0)	ND	4+
	1500 U	13.8	54.0 ± 12 (84.0)	25.3 ± 5.0 (7.7)	ND	2+
($10^{8.3}$ U/mg protein)	5000 U	49.5	58.5 ± 8.5 (96.0)	23.5 ± 0.7 (2.9)	ND	4+
	2500 U	30.1	53.4 ± 9.2 (82.4)	28.5 ± 3.9 (16.2)	ND	4+
	1250 U	0	46.0 ± 6.8 (62.8)	22.5 ± 0.9 (0.3)	ND	4+
	625 U	0	25.5 ± 0.7 (8.2)	21.5 ± 4.9 (0)	ND	-
β-endorphin	5 μg	100	49.3 ± 9.2 (71.5)	26.8 ± 7.1 (11.7)	ND	3+
Morphine sulfate	10 μg	100	60.0 ± 0 (100)	21.0 ± 10.6 (0)	ND	4+
	3.3 μg	71.1	59.5 ± 1.0 (98.7)	22.2 ± 3.5 (0)	ND	4+
	1 μg	56.2	29.1 ± 3.0 (17.8)	ND	ND	1+
Balanced salt solution (injected)		0	22.4 ± 2.2 (0)	ND	ND	-
(noninjected)		-	22.9 ± 2.3 (1.3)	ND	23.0 + 2.0 (1.6)	-

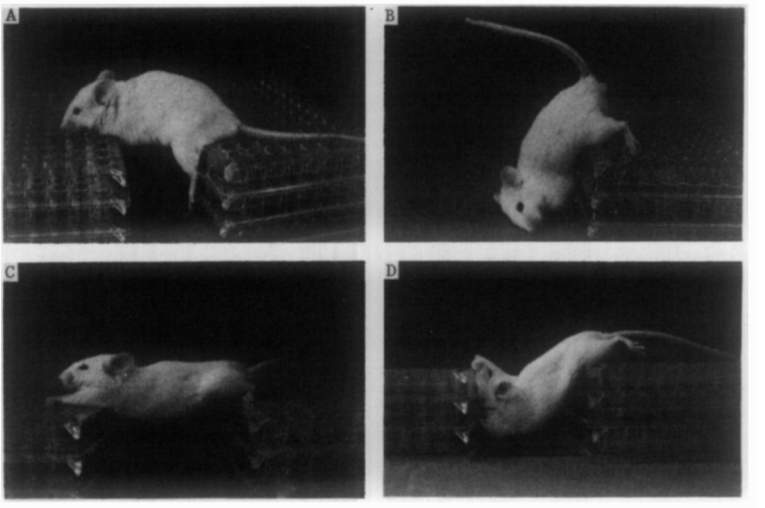

Figure 1. Mice following intracerebral injection of HuIFN-α (500 U) which demonstrate the waxy flexibility and rigid catatonic states characteristic of β-endorphin (A-D). Mice were photographed 5 min following injection on Tri-X film (Kodak, Rochester, New York) at 1/30 sec. The undisturbed mouse in photographs C and D remained as positioned in C for 20 min. At one point, the mouse slid into an awkward position between the holders (D) and remained that way until repositioned.

prevented or reversed the analgesic effect when given before or after HuIFN-α, respectively. This pattern is seen with β-endorphin and morphine and shows that the effect is probably mediated through opiate receptors (Table 1).

Within 1 to 2 minutes following HuIFN-α injection profound behavioral changes are observed. These are characterized by a lack of spontaneous movement (without motor paralysis since movements were possible when animals were startled) and catalepsy (Table 1). The cataleptic state was exhibited by "waxy flexibility" and maintenance of awkward positions (Fig. 1). Naloxone given intraperitoneally (1 mg/kg) immediately reversed all of the behavioral changes and no "wet dog" shakes were observed. These effects lasted for a period of 15 to 30 minutes following injection of HuIFN-α.

DISCUSSION

The observation that HuIFN-α has potent endorphin-like opiate activity suggests that this effect may be related to the lassitude and malaise

experienced during HuIFNα therapy in humans (10). These side effects are two of the most profound and frequently observed. Varicella-zoster patients also report a decrease of pain after HuIFN-α treatment (11). Additionally, personality changes have been observed, especially in children who became quiet, withdrawn and non-communicative (12). A possible relationship between HuIFN-α's opiate like activity and side effects is strengthened by our finding of analgesia in mice which occur about 30 minutes after the intraperitoneal injection of HuIFN-α. Presently, clinical trials are beginning which may involve massive doses of HuIFN-α. It seems important to be aware of possible detrimental effects of HuIFN-α which are mediated through opiate receptors. Control of these side effects by naloxone may be possible since the action of IFN-α on brain-tissue apparently operates through opiate receptors which are probably different from the "classical" IFN receptor. The most compelling evidence for this is a lack of naloxone interference with the antiviral and anticellular activity of HuIFN-α (unpublished data). Different receptors are also suggested by the action of HuIFN-α on mouse behavior while its receptor for antiviral and antitumor activities are more species specific (i.e., primarily demonstrable in humans and their cells). Further evidence for the involvement of different receptors is our finding that mouse interferon from lymphocytes but not fibroblasts shows endorphin-like opiate activity.

These data also provide further evidence that there is a regulatory circuit between the immune and neuroendocrine systems. For instance, in response to infections, tumors or chemicals, lymphocytes may signal the brain or other organs and glands by the production of neuroendocrine or neuroendocrine-like hormones (some of which are associated with HuIFN-α and possibly other lymphocyte products). The high potency of HuIFN-α on the nervous system suggests only minute amounts might be needed to trigger the system. These levels are probably achievable during infections. Thus this putative immune to neuroendocrine regulatory circuit offers a possibly new homeostatic control as well as an etiologic site for psychopathological states.

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