Vol. 104, No. 1, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1982 Pages 341-346

ABSENCE OF FUNCTIONAL AND STRUCTURAL HOMOLOGY OF NATURAL AND RECOMBINANT HUMAN LEUKOCYTE INTERFERON (IFN-α) WITH HUMAN α-ACTH AND β-ENDORPHIN

Lois B. Epstein, Mary E. Rose, Nancy H. McManus and Choh Hao Li

Cancer Research Institute, Department of Pediatrics,
and Hormone Research Laboratory
University of California, San Francisco, California 94143.

Received December 3, 1981

SUMMARY: A comparison of the amino acid sequence of one human recombinant IFN-α (IFLrA) with either human β-endorphin or ACTH reveals only a minimal and insignificant degree of homology. Also, synthetic ACTH, β-endorphin and β-endorphin-(1-15) have no antiviral protective effects on human fibroblasts and cannot inhibit the neutralization of the antiviral effects of natural IFN-α by an antiserum directed against the interferon. Anti ACTH and Anti β-endorphin do not neutralize the antiviral effects of IFLrA, and radioimmunoassays of partially purified natural IFN-α and pure IFLrA do not reveal any evidence of α-MSH or β-endorphin-like material in the interferons. These results demonstrate an absence of functional and structural homology of natural and recombinant IFN-α with ACTH and β-endorphin.

INTRODUCTION

Other investigators have recently suggested that human IFN-α has structural and biological relatedness to human ACTH and the human endorphins (1,2). They reported that anti α-melantropin (α-MSH) and anti γ-endorphin sera neutralize natural human leukocyte interferon (IFN-α) activity and that the neutralization of the interferon by antiserum to it was partially blocked by ACTH. These reports were received with considerable interest since these observations, if substantiated, would expand even further the broad range of action of interferon, open up new areas for study concerning the interrelationship of mechanisms of action of hormones and interferon and possibly provide an explanation for the clinical observation that patients with herpes zoster have a marked decrease in pain when treated with natural IFN-α (3,4). The present studies were performed in an attempt to substantiate the original reports (1,2). However, using a combination of structural, biological and immunological analyses, we were not able to confirm the observations reported by the other investigators.

0006-291X/82/010341-06$01.00/0

MATERIALS AND METHODS

Natural human IFN-α was obtained from the laboratory of Dr. Kari Cantell at the Central Public Health Laboratory in Helsinki, Finland. It was induced in human leukocytes by Sendai virus and partially purified to a specific activity of $4x10^6$ I.U./mg (5).

Human IFN-α prepared by recombinant DNA technology (IFLrA) was obtained from the laboratory of Dr. Sidney Pestka. It was purified to homogeneity by Dr. Donna Hobbs and Dr. Hsiang-fu Kung of the Roche Institute of Molecular Biology in Nutley, New Jersey (6), and its amino acid sequence has been elucidated (7-9).

Antiserum prepared in sheep against Sendai virus induced IFN-α (10) was obtained from Dr. Kari Cantell. It contains 450,000 neutralizing units/ml and was employed in experiments to neutralize the antiviral effects of IFN-α.

Human β-endorphin, a pure synthetic product identical to the natural product, was prepared by the solid phase method (11).

Human β-endorphin-(1-15), a pure synthetic product fully characterized as to the amino acid sequence corresponding to the first 15 amino acids of human β-endorphin, was prepared by the solid phase method (12).

Alpha human ACTH, a pure synthetic product identical to the natural product, was prepared by the solid phase method (13).

Antiserum to human β-endorphin was prepared in rabbits against the synthetic human β-endorphin (14) and has a final dilution of 1/3000.

Antiserum to human α-ACTH was prepared in rabbits (15) and has a final dilution of 1/30,000.

Antiserum to human α-MSH was prepared in rabbits by the technique described by Rao et al. (15), and its final dilution is 1/30,000.

Radioimmunoassays for human β-endorphin and for α-MSH were performed by the method of Chang et al. (14).

An inhibition of cytopathic effect microassay, quantitated by measuring dye elution by a vertical light path photometer, was employed to detect the antiviral protective effects of interferon (16). Human fetal lung fibroblasts, trisomic for chromosome 21 and more sensitive to the antiviral effects of α, β and γ interferons than their matched diploid counterparts (17), were used in the assay.

RESULTS AND DISCUSSION

A comparison of the amino acid sequence of the recombinant IFN-α with that of human β-endorphin reveals that the amount of structural homology is minimal and inconsequential. In the first 17 amino acids of each molecule there are only 2 amino acids in homologous positions. There are only 8 doublet pairs of amino acid neighbors in the interferon which are also found in the β-endorphin, and 1 triplet amino acid sequence, threonine-proline-leucine. However there is no correspondence in the location of the doublets between the two molecules.

Similarly, minimal structural homology was noted between the recombinant IFN-α and ACTH. Only 14 doublet pairs of amino acid neighbors were shared by the interferon and ACTH, but there was no correspondence in the location of the doublets in each molecule. This insignificant degree of structural homology is in keeping with the lack of biologic and immunologic homology which we have found in all of our experiments.

TABLE 1

LACK OF ANTIVIRAL EFFECT OF ACTH, β_h-ENDORPHIN AND β_h-ENDORPHIN-(1-15) AS COMPARED WITH NATURAL IFN-α

Substance	Concentration (units/ml)	Percent Dye Uptake
Natural IFN-α	6.3	78%
	3.1	58%
	1.6	31%
α_h-ACTH	(5-0.16)*	0%
β_h-Endorphin	(5-0.16)*	0%
β_h-Endorphin-(1-15)	(5-0.16)*	0%

* 1 unit = 0.01 mg.

As it had been reported by others that ACTH had shown interferon-like antiviral activity (2), we tested our synthetic ACTH and β-endorphin for their antiviral protective effects on trisomy 21 human fetal lung fibroblasts against a challenge with bovine vesicular stomatitis virus. As can be seen from the data in Table 1, good antiviral protection was exhibited by the natural IFN-α, but synthetic human ACTH, β-endorphin, and β-endorphin-(1-15), each in a concentration range of 5 to 0.16 units/ml, had no antiviral protective effects.

In our next series of experiments we examined the question of whether synthetic ACTH or synthetic endorphins could inhibit the neutralization of the antiviral effects of natural IFN-α by an antiserum directed against IFN-α. As can be seen from the data in Table 2, 81% of the IFN-α was neutralized by treatment with a dilution of anti IFN-α serum chosen to avoid antibody excess.

TABLE 2

INABILITY OF α_h-ACTH, β_h-ENDORPHIN AND β_h-ENDORPHIN-(1-15) TO BLOCK NEUTRALIZATION OF NATURAL IFN-α BY ANTISERUM TO IFN-α

Substance Added	Concentration (units/ml)	Percent Neutralization of IFN-α*
None	-	81%
ACTH	(1.25-0.001)	>81%
β_h-Endorphin	(1.25-0.001)	>81%
β_h-Endorphin-(1-15)	(1.25-0.001)	≥81%

TABLE 3

INABILITY OF ANTI α_h-ACTH TO NEUTRALIZE THE ANTIVIRAL EFFECTS OF RECOMBINANT IFN-α

Components of System	Per cent Dye Uptake
IFLrA	35%
IFLrA + Anti IFN-α	0%
IFLrA + 1/100 Anti ACTH	40%
IFLrA + 1/500 Anti ACTH	38%
IFLrA + 1/1000 Anti ACTH	33%
IFLrA + 1/5000 Anti ACTH	32%

When the anti IFN-α was pretreated with varying concentrations of synthetic ACTH or synthetic endorphins prior to combining it with the IFN-α, neutralization of the IFN-α was not inhibited, with values of 81% or more observed. In an additional experiment in which a higher concentration of ACTH was employed than that shown in Table 2, and varying dilutions of anti IFN-α capable of neutralizing 6-100 units were also employed, no interference with the neutralization by ACTH was noted.

We then performed experiments to determine if anti human α-ACTH serum could neutralize the antiviral effects of our pure recombinant IFN-α, and the data are shown in Table 3. In the presence of the recombinant IFN-α we

TABLE 4

INABILITY OF ANTI β_h-ENDORPHIN TO NEUTRALIZE THE ANTIVIRAL EFFECTS OF RECOMBINANT IFN-α

Components of System	Per cent Dye Uptake
IFLrA	22%
IFLrA + 1/100 Anti β-Endorphin	21%
IFLrA + 1/500 Anti β-Endorphin	29%
IFLrA + 1/1000 Anti β-Endorphin	21%
IFLrA + 1/5000 Anti β-Endorphin	24%

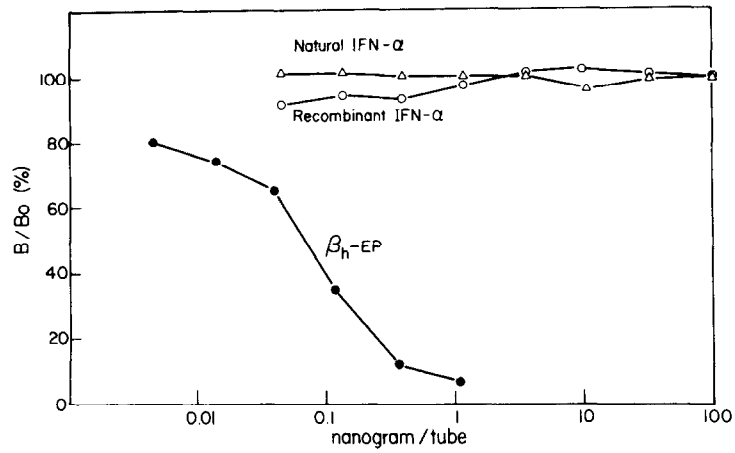

FIG. 1: Radioimmunoassay of natural and recombinant IFN-α for human β-endorphin. The vertical axis reflects the per cent specific binding of [^{125}I]-human β-endorphin. The horizontal axis reflects the amount of cold β-endorphin or natural or recombinant IFN-α added.

observed a dye uptake of 35%. The antiviral activity of this recombinant IFN-α was completely neutralized by antiserum to natural IFN-α. However, no neutralization of the antiviral effects of the recombinant IFN-α was noted with dilutions of anti ACTH serum ranging from 1/100-1/5000.

Similar experiments were performed with an antiserum to human β-endorphin (Table 4). In dilutions ranging from 1/100-1/5000 it also did not neutralize the antiviral effects of recombinant IFN-α.

Radioimmunoassays for human β-endorphin and α-MSH were performed on our natural IFN-α and pure recombinant IFN-α, and the data for the radioimmuno-

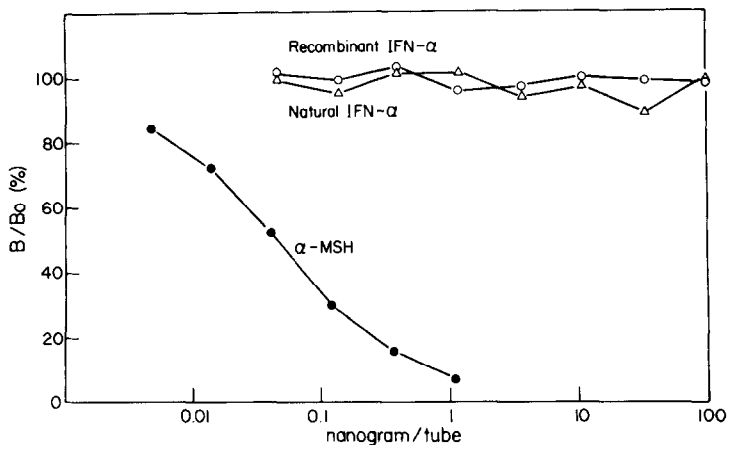

FIG. 2: Radioimmunoassay of natural and recombinant IFN-α for human α-MSH. The vertical axis reflects the per cent specific binding of [^{125}I]-human α-MSH. The horizontal axis reflects the amount of cold α-MSH or natural or recombinant IFN-α added.

assay for β-endorphin are depicted in Figure 1. It is apparent that there is no β-endorphin-like material in either the natural or recombinant IFN-α.

The data from a similar experiment in which a radioimmunoassay of the natural and recombinant IFN-α preparations for α-MSH was performed are depicted in Figure 2. It is apparent that no α-MSH-like material was observed.

In conclusion, we have demonstrated that only minor and insignificant structural homology exists between one form of recombinant IFN-α and human β-endorphin or human ACTH and that no biologic or immunologic homology exists between natural or a recombinant form of IFN-α and human β-endorphin and ACTH.

ACKNOWLEDGEMENTS

This work was supported by NIH grants CA27903 and GM2907 and by March of Dimes Birth Defects Foundation grant 6-126. We thank Mary Lou Sumberg for typing the manuscript.

REFERENCES

1. Blalock, J.E. and Smith, E.M. (1980) Proc. Natl. Acad. Sci. USA 77, 5972-5974.
2. Blalock, J.E. and Smith, E.M. (1981) In The Biology of the Interferon System (E. De Maeyer, G. Galasso and H. Schellekens, eds.), pp. 93-99, Elsevier/North Holland Biomedical Press, Amsterdam.
3. Emodi, G. and Rufli, T. (1977) Tex. Rep. Biol. Med. 35, 511-515.
4. Merigan, T.C., Rand, K.H., Pollard, R.B. et al. (1978) N. Engl. J. Med. 298, 981-987.
5. Cantell, K. and Hirvonen, S. (1978) J. Gen. Virol. 39, 541-543.
6. Staehelin, T., Hobbs, D.S., Kung, H.F. et al. (1981) J. Biol. Chem. 256, 9750-9754.
7. Maeda, S., McCandliss, R., Gross, M. et al. (1980) Proc. Natl. Acad. Sci. USA 77, 7010-7013.
8. Goeddel, D.V., Yelverton, E., Ullrich, A. et al. (1980) Nature 287, 411-416.
9. Wetzel, R., Perry, L.J., Estell, D.A. et al. (1981) J. Interferon Res. 1, 381-390.
10. Mogensen, K.E., Pyhala, L. and Cantell, K. (1975) Acta Path. Microbiol. Scand. 83, 443-450.
11. Li, C.H., Yamashiro, D., Tseng, L.F. et al. (1977) J. Med. Chem. 20, 325-328.
12. Yeung, H.W., Yamashiro, D., Chang, W.C. et al. (1978) Int. J. Peptide Protein Res. 12, 42-46.
13. Yamashiro, D. and Li, C.H. (1973) J. Amer. Chem. Soc. 95, 1310-1315.
14. Chang, W.C., Yeung, H.W. and Li, C.H. (1979) Int. J. Peptide Protein Res. 13, 278-281.
15. Rao, A.J., Long, J.A. and Ramachandran, J. (1978) Endocrinol. 102, 371-378.
16. Weil, J., Epstein, L.B. and Epstein, C.J. (1980) J. Interferon Res. 1, 111-124.
17. Epstein, L.B. and Epstein, C.J. (1976) J. Inf. Dis. 133, Suppl. A56-A62.