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HUMAN INTERFERON ENHANCES GUNAYLATE CYCLASE ACTIVITY

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SUMMARY: Partially purified human leukocyte interferons, partially purified human lymphoblastoid interferon, and human fibroblast interferon enhanced rat liver, kidney, and splenic guanylate cyclase {E.C.4.6.1.2.} activity 2-4 fold at 5 μIU concentration. Dose-response relationships revealed that the human leukocyte interferons enhanced splenic guanylate cyclase activity at concentrations as low as 0.01 μIU while a concentration of 1 μIU for partially purified human lymphoblastoid interferon and 10 μIU concentration for human fibroblast interferon were necessary to see any effect on guanylate cyclase activity.

INTRODUCTION: Interferon in recent years has been reported to have anti-tumor, as well as anti-viral effects (1,2). Interferon's mechanism of action for these anti-tumor effects remains unknown. Since some anti-cancer agents such as streptozotocin appear to have part of their mechanism of action through the stimulation of guanylate cyclase {GTP pyrophosphate-lyase (cyclizing), E.C.4.6.1.2.} (3,4), the effect of partially purified human leukocyte interferons were tested on the guanylate cyclase-cyclic GMP system. These interferons, as well as human lymphoblastoid interferon and human fibroblast interferon, all had stimulatory effects on splenic guanylate cyclase activity.

METHODS

Interferons: The human leukocyte interferon was prepared as previously described (5,6). Crude interferon had an activity of 40,000 international units (IU)/ml. One unit of activity is the reciprocal of the dilution that inhibits virus plaque formation by fifty percent. All units are expressed

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in terms of the international reference preparation 69/19(7). It was concentrated 30-fold, by precipitation with acid KSCN, to give a preparation containing $1x10^6$ IU/ml and 50 mg of protein/ml. This interferon is called crude concentrated interferon (C-IF) in the text. Partial purification of the human leukocyte interferon was done with selective precipitation of inert proteins from ethanolic solution with rising pH, to a specific activity of ca. $1x10^6$ IU/mg of protein (6). The partially purified interferons A (P-IF A), B (P-IF B) and C (P-IF 147-161) contained $6x10^6$ IU/ml and 5, 7.3, and 1.5 mg of protein/ml respectively. Mock interferon was prepared by treating leukocyte suspensions as in the routine production of human leukocyte interferons, but normal allantoic fluid was added rather than Sendai virus. The partially purified mock interferon contained < 200 IU/ml and 22 mg of protein/ml.

The partially purified human lymphoblastoid interferon was a gift from Dr. N.B. Finter, The Wellcome Research Laboratories. It contained $5x10^6$ IU/ml and 7.5 mg protein/ml. Human fibroblast interferon was a gift from Dr. J. Vilcek, New York University. It contained $2x10^6$ IU/ml and 5 mg protein/ml.

Guanylate Cyclase Assay: Tissues used in these experiments were obtained from male 150-200 g Sprague Dawley rats that had been maintained ad libitum on Purina Laboratory Chow. All dilutions of the various interferon preparations were made in triple-distilled water. Guanylate cyclase activity was measured as previously described (3,4,8). The various tissues were homogenized in cold 0.03 M Tris HCl, pH 7.6, and centrifuged at 37,000 g at 4^0 C for 15 minutes. The supernatant, to which the above interferons has been added to obtain the final concentrations noted in the text, was then assayed at 37^0 C for 10 minutes for guanylate cyclase activity. The reaction mixture consisted of 20 mM Tris HCl, pH 7.6; 4 mM $MnCl_2$; 2.67 mM cyclic GMP (used to minimize destruction of cyclic {^{32}P}-GMP); a GTP regenerating system (5 mM creatine phosphate, 11.25 U creatine phosphokinase, E.C.2.7.3.2.); 100 µg bovine serum albumin; 20 mM caffeine; 1.2 mM {α^{32}P}-GTP, approximately 5 x 10^5 cpm; and the enzyme preparation having 0.2 to 0.4 mg protein. The final pH of the reaction mixture was 7.6. The volume of supernatant fraction was 25λ and the final volume of the cyclase assay, which includes the above supernatant fraction, the reaction mix, and the radioactive isotopes, was 75λ. After a 10-minute incubation, the reaction was terminated by the addition of 10 µl of 0.1 M EDTA, pH 7.6, containing about 30,000 cpm of cyclic {^{3}H}-GMP (to estimate recovery in the subsequent steps) and boiling for three minutes. After cooling in an ice bath, the cyclic {^{32}P}-GMP formed was isolated by sequential chromatography on Dowex-50-H^+ and alumina using the modification described in detail previously (8). The overall recovery of cyclic GMP after the two-stage chromatographic procedure was 95%. Blank {^{32}P}-counting rates averaged 40-50 cpm. With this assay system, production of cyclic GMP was linear with time for at least 20 minutes and with added protein from 50 to 400 µg. All of the {^{32}P}-containing material was identifiable as cyclic GMP as determined by thin-layer chromatography on PEI-cellulose (Brinkman, Westbury, N.Y.) using 1 M LiCl as solvent and on Chromar sheets (Mallinckrodt Chemical Works, St. Louis, MO) developed with absolute alcohol and concentrated NH_4OH (5:2 v/v). Each of the respective interferons was added to the supernatant without any preincubation. Each assay was conducted in triplicate and the results confirmed in three separate experiments.

RESULTS

A 30-fold concentration of human leukocyte interferon called crude concentrated human leukocyte interferon (C-IF) enhanced guanylate cyclase

TABLE I

Human Leukocyte Interferons, Human Lymphoblastoid Interferon and Human Fibroblast Interferon Enhance Guanylate Cyclase Activity

Addition, 5 μIU	Cyclic GMP (pmoles/mg protein/10 min. incubation)* Spleen	Kidney	Liver
None	609±19	290±12	284±6
Crude leukocyte interferon	1305±16†	586±16†	602±8†
C-IF	1522±30†	605±13†	647±10†
P-IF A$^{\infty}$	1827±17†	888±10†	903±19†
P-IF B$^{\infty}$	2632±18†	1203±16†	987±17†
P-IF 147-161$^{\infty}$	2397±22†	1193±12†	1003±8†
Lymphoblastoid$^{\infty}$	1799±19†	901±6†	831±16†
Fibroblast	1297±12†	597±8†	609±13†

* Each value is the mean ± S.E.M. of triplicate samples done in six animals in group. The enzyme activity is expressed as picomoles of cyclic GMP formed per milligram of protein per 10 minute incubation. Assay conditions are as described in the Methods.

† Significant at $P<0.001$ compared to control by Student t test for unpaired values.

^{ns} Not Significant

$^{\infty}$ CIF = crude concentrated human leukocyte interferon; PIF-A, B, and 147-161 = partially purified human leukocyte interferons A, B, and 147-161; lymphoblastoid = partially purified human lymphoblastoid interferon.

activity two-fold in liver, kidney, and spleen at a concentration of 5 μIU/ml (Table I). Further purification of human leukocyte interferon has resulted in three partially purified human interferons (PIF-A, PIF-B, and PIF-147-161) all of which enhanced guanylate cyclase activity in the same tissues at the same concentrations (Table I). This enhancement was four-fold with PIF-B and PIF-147-161, while the enhancement was three-fold with PIF-A in kidney and spleen. In liver, PIF-A, PIF-B, and PIF-147-161 all had a similar three-fold enhancement of guanylate cyclase activity. Mock leukocyte interferon at concentrations as high as 2 μg of protein/ml caused no enhancement of

TABLE II

Dose-Response Relationships of the Various Interferons on Splenic Guanylate Cyclase Activity

Addition μU/ml	Cyclic GMP (pmoles/mg protein/10 minute incubations)* PIF-A$^{\infty}$	PIF-B$^{\infty}$	PIF-147$^{\infty}$	LBI$^{\infty}$	FI$^{\infty}$
0	611±17	603±13	601±10	608±15	605±8
.001	604±12^{ns}	621±10^{ns}	616±17^{ns}	618±12^{ns}	607±15^{ns}
.01	705±23^{ns}	1283±17†	1197±12†	621±14^{ns}	611±13^{ns}
.1	1207±17†	1987±21†	1684±20†	651±33^{ns}	622±12^{ns}
1	1348±12†	2599±8 †	1983±16†	1335±13†	659±21^{ns}
10	1811±20†	2672±12†	2401±23†	1833±12†	1211±13†
100	1832±19†	2849±10†	2413±17†	1844±11†	1293±10†
1000	1847±15†	1832±13†	2399±21†	1794±16†	1303±14†

* Each sample is the mean ± S.E.M. of triplicate samples on each of six animals each group. Assay conditions are as described in the Methods.

† Significant at $P<0.001$ compared to control by Student t test for unpaired values.

∞ PIF-A, B = partially purified human leukocyte interferons A & B; PIF-147=partially purified human leukocyte interferon 147-161; LBI=partially purified human lymphoblastoid interferon; FI=human fibroblast interferon.

ns Not Significant.

guanylate cyclase activity. All the true interferon preparations tested caused enhancement of guanylate cyclase activity at this concentration of protein and at 1000 fold lower concentrations.

Dose-response curves for the patially purified leukocyte interferons, human fibroblast interferon, and partially purified human fibroblast interteron are shown in Table II. PIF-B and PIF-147-161 enhanced splenic guanylate cyclase activity at concentrations as low as 0.01 μIU and had a near maximal enhancement of 10 μIU. There was no effect on splenic guanylate cyclase activity with crude human leukocyte interferon until a concentration of 1 μIU was reached (not shown). Maximal stimulation of guanylate cyclase

activity was seen at 10 μIU with all the partially purified human leukocyte interferons and no further enhancement was seen with raising the concentration to as high as 8,000 IU. Lymphoblastoid interferon did not have an effect on splenic guanylate cyclase activity below a concentration of 1 μIU while human fibroblast interferon needed a concentration of 5 μIU to have an effect (Table II).

DISCUSSION

The data in the present investigation demonstrates that human leukocyte, fibroblast, and lymphoblastoid interferons can stimulate guanylate cyclase activity. Since our original description in abstract form of the human interferons enhancing guanylate cyclase activity (9) there has been a report of interferon isolated from mouse sarcoma C243-3 cells increasing cyclic GMP concentration in cell culture 5-10 minutes after addition to the culture at a concentration of 1 unit/10^3 cells (10). This finding correlates well with findings of the human interferons enhancing guanylate cyclase over a similar period of time. It should be noted that the above report (10) and our own studies have been done with partially purified interferons and the effects seen on the guanylate cyclase-cyclic GMP system might be due to a contaminant. The increases in cyclic GMP did not correlate with the potency of the various interferons. The proof of whether interferon's mechanism of action involves the guanylate cyclase-cyclic GMP system awaits complete purification of interferon and then further investigation with the guanylate cyclase-cyclic GMP system.

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