PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 501-504 (1976)

Inhibition of Interferon Action by Cytochalasin B, Colchicine, and Vinblastine (395578)

M. F. BOURGEADE AND C. CHANY

Institut National de la Santé et de la Recherche Médicale, U.43, Hôpital St. Vincent de Paul, 74 avenue Denfert-Rochereau, Paris 75014, France

We have previously postulated that a membrane-bound receptor system is responsible for the initial steps leading to the antiviral protection induced by interferon (1). Additional experiments using insoluble interferon (2) or ouabain (3) support this hypothesis. The role of the receptor system is also substantiated by the observation that antibodies directed against interferon block the establishment of the antiviral state in interferon-producing cells (4). The dose-response curves using either soluble or insoluble interferon are sigmoidal, thus suggesting a cooperative event in antiviral action. This cooperative event could occur at the receptor level as shown by studies using all hybrids (1, 5).

Since cooperative activation could involve individual receptors or a number of them, we wondered whether the mobility of such receptors is important or not in interferon action. The microtubule and microfilament systems, among other properties (6), have been associated with the mobility of membrane-bound proteins (7). It was therefore of interest to study the effect on interferon action of cytochalasin B, colchicine, and vinblastine, which are known to disrupt these systems (8, 9).

Materials and methods. Mouse L929 cells were cultivated in 35-mm petri dishes (7×10^5 cells per dish) in Eagle's minimum essential medium (MEM) containing 10% calf serum. Murine interferon was produced in LM cells induced by Newcastle disease virus (NDV) following standard procedures. The preparations used in these experiments contained 600 interferon reference units (sp act: 2.5×10^4 units/mg of protein). The L929 cells were treated with interferon and simultaneously with cytochalasin B (Calbiochem), colchicine (Sigma), or vinblastine sulfate (Sigma), alone or in combination at the final concentration of 5 μg/ml. After 4 hr of incubation, the medium and drugs were removed and cells were challenged with encephalomyocarditis virus (EMC) at a multiplicity of infection (m.o.i.) = 1 for 1 hr. The viral yield was measured by plaque titration after 18 hr of incubation at 37°.

Effect of the drugs on cellular RNA and protein synthesis was estimated as follows: cells were labeled with ^{14}C-amino acid (protein hydrolysate of *Chlorella vulgaris,* sp act 810 μCi/mg) at a concentration of 0.2 μCi/ml, and [^{3}H]uridine (sp act 25 Ci/mmole) at a concentration of 1 μCi/ml (both purchased from C.E.A. Saclay, France), which were added to confluent monolayers of L cells. Cytochalasin B, colchicine, or vinblastine were added simultaneously at a concentration of 5 μg/ml. After 4 hr of incubation at 37°, cells were washed three times with cold PBS, then 0.3% SDS was added for 20 min at 37°. TCA (10%) was added for 1 hr at 0°, and after centrifugation, the precipitate was washed with 5% TCA and dissolved in ammonium. Both the acid-soluble and insoluble fractions were counted using Bray's scintillator fluid.

Results and discussion. As shown in Table I, when L cells were treated simultaneously with murine interferon and either cytochalasin B, colchicine, or vinblastine, all three drugs significantly decreased the antiviral properties of interferon. However, the association of cytochalasin B either with colchicine or vinblastine inhibited even to a greater extent the antiviral action of interferon. The association of vinblastine and colchicine was less efficient.

Since for interferon action the integrity of cell protein synthesis is requisite, it was of importance to find out whether or not such a modification of the cell metabolism could explain the observed results. Therefore in a parallel series of experiments, we explored the effect on RNA and protein synthesis of

Copyright © 1977 by the Society for Experimental Biology and Medicine
All rights reserved.

these three drugs, used alone or in combination. As shown in Table II, during the 4-hr duration of the experiment at the concentration employed, they did not produce detectable changes in the incorporation of ^{14}C-labeled amino acid into the cell.

In further experiments, when cells were labeled with [^{3}H]uridine in the presence of the different drugs, no significant change in [^{3}H]uridine incorporation was observed either in the cellular pool or in the acid-insoluble fraction, except in the case of cytochalasin B. When this drug was added to the cells in the presence of labeled uridine for 4 hr, a 50% decrease in uridine incorporation into RNA was observed. Since a similar decrease was noted in the acid-soluble fraction, this reflected in fact a diminution in the intracellular pool of [^{3}H]uridine. When the cells were labeled for 5 min and then washed prior to the addition of the drug, uridine incorporation in the acid-insoluble compartment was similar to untreated cells (Fig. 1). Thus, cellular RNA synthesis is not really involved in the anti-interferon effect of cytochalasin B.

Likewise, the inhibition of glucose uptake by cytochalasin B is probably not the explanation of the decreased response of the cells to interferon. The absence of glucose in the medium during the experiment did not affect the establishment of the antiviral state (unpublished experiments).

Previous investigations on the mechanism

TABLE I. EFFECT OF CYTOCHALASIN B, COLCHICINE, AND VINBLASTINE ON INTERFERON ACTION.[a]

	Experiment 1		Experiment 2		Experiment 3	
	IF	MEM	IF	MEM	IF	MEM
Control	2.7×10^6	6.2×10^8	10^5	2.3×10^8	5.6×10^6	8×10^8
Cytochalasin B	2.5×10^7	7×10^8	2×10^6	3×10^8	2×10^7	1×10^9
Colchicine	1.7×10^7	4.6×10^8	–	–	2.7×10^7	3.3×10^9
Vinblastine	–	–	4×10^6	2.6×10^8	3.5×10^7	9×10^8
Cytochalasin B + colchicine	6.3×10^7	5×10^8	–	–	3×10^7	7.6×10^8
Cytochalasin B + vinblastine	–	–	1.3×10^7	1.5×10^8	7.2×10^7	7×10^8
Colchicine + vinblastine	–	–	–	–	4.4×10^7	8×10^8

[a] L cells were treated with interferon (IF) and simultaneously with cytochalasin B, colchicine, and vinblastine, alone or in combination. After 4 hr of treatment, interferon and drugs were eliminated and cells were infected with EMC. The viral yield is expressed in plaque-forming units per milliliter. The standard error of the titration measured after 10 independent estimations of the same suspension is ±0.11 log for $P < 0.01$.

TABLE II. EFFECT OF CYTOCHALASIN B, COLCHICINE, AND VINBLASTINE ON CELLULAR RNA AND PROTEIN SYNTHESIS.[a]

	[^{3}H]Uridine		^{14}C-labeled amino acids	
	Acid soluble fraction (324,376)[d]	Acid-insoluble fraction (121,041)[d]	Acid-soluble fraction (14,498)[d]	Acid-insoluble fraction (6368)[d]
Cytochalasin B	58.66[b] ± 10.38[c]	62.8[b] ± 13.13	101.2 ± 13.77	99.2 ± 2.82
Colchicine	97.6 ± 2.85	103.4 ± 12.33	98.4 ± 9.77	100.8 ± 8.72
Vinblastine	99.66 ± 13.65	101.33 ± 12.23	93.33 ± 4.60	98.33 ± 10.32
Cytochalasin B + colchicine	53.5[b] ± 10.49	58[b] ± 13.46	93.60 ± 4.42	103.4 ± 12.17
Cytochalasin B + vinblastine	70.5[b] ± 6.42	71.5[b] ± 2.75	92 ± 2.42	90.5 ± 8.25
Vinblastine + colchicine	96.66 ± 2.85	100.33 ± 2.48	95.33 ± 13.01	95.66 ± 11.93

[a] ^{14}C-labeled amino acid and [^{3}H]uridine incorporation in cellular RNA and protein was measured as described in Materials and Methods. The results are expressed in percentage as compared to the control and calculated on the basis of three independent experiments.

[b] Statistically different from the control for $P < 0.01$.

[c] Each entry represents mean ± SE for $P < 0.05$.

[d] cpm in control cells (mean of three experiments).

of action of the three drugs here studied have shown that they are able to modify cellular response to lectins in the absence of any detectable competition for binding sites. In the case of both leukocytes (10) and transformed cells (11), colchicine or vinblastine decreased cell agglutination produced by concanavaline A. Similar findings were obtained with cytochalasin B (12). The authors suggested that topographic modifications in the membrane-bound receptors induced by the drugs could explain their results. In addition, using freeze-etch electron microscopy, a modification in the distribution of membrane-intercalating particles was observed after treatment with colchicine (13).

In our studies, although the drugs were effective alone, their potency increased when associated. Blockage of interferon action was always obtained in the absence of any detectable modification of cellular RNA or protein synthesis. This view is also supported by the observation that at concentrations here employed, colchicine and vinblastine (14) and cytochalasin B (15) do not decrease interferon synthesis. Thus, the integrity of microfilaments and microtubules seems to be necessary for the induction of the antiviral effect by interferon. They could either modify the stability of membrane-bound interferon receptors or prevent an association between receptors or other components requisite for the initiation and amplification of the cellular response to this substance. Likewise, the disruption of microtubules could interfere with receptor–cytoplasmic interactions as previously suggested (16–18).

It is unlikely, however, that the known modifications in the active uptake of glucose due to cytochalasin B could explain our results. The observed decrease of uridine uptake is probably also not involved in the loss of the antiviral effect of interferon. In our experimental conditions, no change in the amino acid uptake is noted in contrast to a previous report (19), perhaps because of the smaller drug concentrations employed.

It can be concluded from our studies that the drug-induced decrease of interferon activity is due to modifications of the cell membrane structure. This could be related to the inhibitory effect of ouabain on the interferon-induced antiviral state (3). It is also possible, however, that other presently unknown properties of these inhibitors could contribute to explain these results.

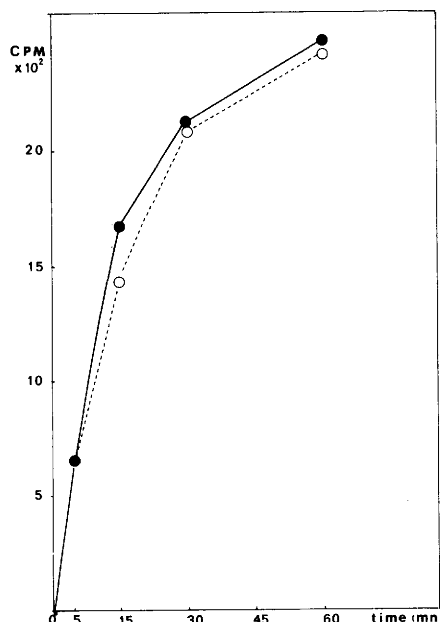

FIG. 1. Incorporation of [^{3}H]uridine into RNA in the presence of cytochalasin B. [^{3}H]Uridine (5 μCi/ml) was added to L cells for 5 min at 37°. After removal of uridine and three washings with MEM, cytochalasin B (5 μg/ml) was added. At different time periods, cells were washed in cold PBS and treated as described in Methods. ●——● MEM; ○- - -○ cytochalasin B.

Summary. Cytochalasin B, colchicine, and vinblastine decrease the antiviral effect of interferon when added to cells simultaneously with interferon. The drugs are effective alone but their potency increases when used in combination. The lack of development of the antiviral state is observed without any detectable modifications in cellular RNA or protein synthesis. These drugs are known to disrupt microtubules and microfilaments which are therefore probably necessary for interferon action. They could be involved in the distribution of membrane-associated receptors and/or in interactions between receptors and the cytoplasma. The initial steps leading to the establishment of antiviral protection by interferon could depend on these mechanisms.

We thank Isabelle Tardivel for technical assistance

and Carol Girard for help with the manuscript. Partial support came from the Délégation Générale à la Recherche Scientifique et Technique (Contract No. 74.7.0576).

1. Chany, C., Grégoire, A., Vignal, M., Lemaitre-Moncuit, J., Brown, P., Besançon, F., Suarez, H., and Cassingena, R., Proc. Nat. Acad. Sci. USA **70,** 557 (1973).
2. Ankel, H., Chany, C., Galliot, B., Chevalier, M. J., and Robert, M., Proc. Nat. Acad. Sci. USA **70,** 2360 (1973).
3. Lebon, P., Moreau, M. C., Cohen, L., and Chany, C., Proc. Soc. Exp. Biol. Med. **149,** 108 (1975).
4. Vengris, V. E., Stollar, B. D., and Pitha, P. M., Virology, **65,** 410 (1975).
5. Bourgeade, M. F., Proc. Soc. Exp. Biol. Med. **146,** 820 (1974).
6. Wessels, N. L., Spooner, B. S., Ash, J. F., Bradler, M. O., Luduena, M. A., Taylor, E. L., Wrenn, J. R., and Yamada, K. M. Science **171,** 135 (1971).
7. Poste, G., Papahadjopoulos, D., and Nicolson, G. L., Proc. Nat. Acad. Sci. U.S.A. **72,** 4430 (1975).
8. Marantz, R., Ventilla, M., and Shelanski, M. L., Science **195,** 498 (1969).
9. Borisy, G. G., and Taylor, E. W., J. Cell Biol. **34,** 525 (1967).
10. Berlin, R. D., and Ukena, T. E., Nature New Biol. **238,** 120 (1972).
11. Yin, H. H., Ukena, T. E., and Berlin, R. D., Science **178,** 867 (1972).
12. De Petris, S., Nature (London) **250,** 54 (1974).
13. Wunderlich, F., Müller, R., and Speth, V., Science **182,** 1136 (1973).
14. Havell, E. A., and Vilcek, J., J. Cell Biol. **64,** 716 (1975).
15. Ito, Y., Nishiyama, Y., Shimokata, K., Kimura, Y., Nagata, I., and Kunii, A., Virology **71,** 463 (1976).
16. Edelman, G. M., Yahara, I., and Wang, J. L., Proc. Nat. Acad. Sci. USA **70,** 1442 (1973).
17. Yahara, I., and Edelman, G. M., Exp. Cell Res. **91,** 125 (1975).
18. Chen, K., Heller, J., and Canellakis, E. S., Biochem. Biophys. Res. Commun. **68,** 401 (1976).
19. Fyte, M. J., Loftfield, S., and Goldman, I. D., J. Cell. Physiol. **86,** 201 (1975).

Received March 22, 1976. P.S.E.B.M. 1976, Vol. 153.