Interferon Inhibition by Narcotic Analgesics (36968)

CHUNG Y. HUNG, STANLEY S. LEFKOWITZ,[1] AND WILLIAM F. GEBER

Department of Cell and Molecular Biology and Department of Pharmacology, Medical College of Georgia, Augusta, Georgia 30902

Interferon is believed to play an important role in the recovery from virus infections. It is one of the earliest defense mechanisms marshalled to protect the host from invasion. Although various factors have been demonstrated to affect the circulating level of interferon (1), to our knowledge, the effect of narcotics on the induction of interferon has not been demonstrated. This report presents evidence that administration of the drugs, morphine, Dilaudid, and methadone resulted in a decrease in the level of circulating interferon following injection by poly I:C, a double-stranded, synthetic RNA capable of inducing interferon (2).

Materials and Methods. Mouse L cells (strain 929) were grown in Eagle's MEM containing 10% fetal calf serum (FCS) plus antibiotics. Two percent FCS was used for maintenance of cultures.

Vesicular stomatitis virus, Indiana strain (VSV), was grown in L cells maintained in 32 oz prescription bottles. Virus was harvested by four cycles of freezing and thawing when 75% of the cells showed cytopathic effects. Cell debris was removed by low-speed centrifugation and the supernatant fluid used for virus stock was stored at −20°.

Morphine sulfate was obtained from Merck and Co., Inc. Dilaudid hydrochloride was obtained from Knoll Pharmaceutical Co. Methadone hydrochloride (USP 200 mesh) was obtained from Endo Laboratories. The synthetic double stranded poly I:C was obtained from P-L Biochemicals in a lyophilized form. Bacterial endotoxin (lipopolysaccharide from *Salmonella typhimurium* prepared by the Boivin trichloracetic acid procedure) was obtained from Difco Laboratories.

Random bred male Swiss albino mice were used throughout these studies. Four- to 5-wk-old mice weighing between 25 and 30 g were injected with drugs subcutaneously followed by a single intraperitoneal (ip) injection of poly I:C (1 mg/kg). Six to 8 hr later mice were bled by cardiac puncture and their sera were saved for interferon assay.

Our standard mouse serum interferon was prepared by intraperitoneal injection of poly I:C (2 mg/kg). Six hours later the sera were collected by cardiac puncture. Interferon assay was carried out by the plaque reduction technique and compared with the NIH mouse reference interferon. The titer of our preparation was calculated as 3668 units/ml.

The plaque reduction assay used in these studies was similar to that of Wagner, Levy and Smith (3) and can be summarized as follows: sera from 5 mice containing interferon were pooled in equal amounts, diluted in growth medium, then added to mouse L-929 cells. The cells were incubated 5 hr at 37° in a CO_2 incubator, washed twice with Hanks' balanced salt solution (BSS) and infected with approximately 100 plaque forming units (PFU)/plate of VSV. After absorption for 1 hr at 37°, the cells were washed and incubated for an additional 72–84 hr with maintenance medium containing 1% methyl cellulose. Cells were fixed and stained with 1% crystal violet in 15% ethyl alcohol. Plaques caused by VSV were counted and the titer of interferon was determined by its ability to inhibit 50% of the plaques.

These experiments were divided into two separate areas. First was the effect of these drugs *in vivo* and the second area was the effect of drugs *in vitro*. Primary concern was with the effect of morphine as the representative drug of this group.

[1] Present address: Department of Microbiology, Texas Tech University School of Medicine, Lubbock, TX 79409. Address reprint requests to Stanley S. Lefkowitz.

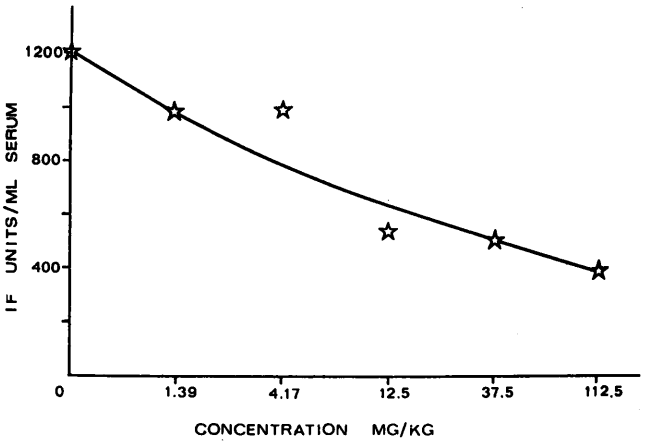

FIG. 1. Dosage response of morphine. Mice were injected with morphine at various concentrations, 4 hr later poly I:C (1 mg/kg) was injected. All mice were bled 6 to 8 hr postinjection of poly I:C. Interferon titer was reported as units/ml of serum.

Results. The behavior of mice injected with either morphine, Dilaudid or methadone was markedly altered. Hyperexcitability was noted for a period of approximately 4 hr in mice injected with these narcotic analgesics. Straub tails were also observed during this period.

Results indicated that administration of these drugs 4 hr prior to a single injection of poly I:C (1 mg/kg) markedly affected the level of circulating interferon. Table I shows that the narcotic analgesics used in this study reduced the interferon titer from 68 to 86% below that of the control. In order to more closely ascertain the level of morphine necessary to markedly inhibit interferon production various concentrations of morphine were utilized as described in Fig. 1. Morphine at a concentration of 1.39 mg/kg of animal reduced the interferon level by 20% compared to 67% using the highest dose. Hyperexcitability was not observed when mice were injected with the lowest dose of morphine. Experiments were designed to determine how long after a single injection of narcotic can poly I:C induction of interferon be affected. Figure 2 illustrates that 72 hr after a single injection of these drugs the animals were still refractory to interferon production. Dilaudid was the most effective in reducing interferon yields which were only 20% of the control even after 72 hr. Further experiments utilizing morphine administered up to 9 days prior to poly I:C caused a 20% reduction of interferon compared to the control.

TABLE I. The Effect of Drugs on the Level of Circulating Interferon Following Intraperitoneal Injection of Poly I:C.[a]

Treatment[b]		Interferon titer[c]
Morphine	112.50 mg/kg	345
Dilaudid	50.00 mg/kg	239
Methadone	15.00 mg/kg	570
Saline	0.15 ml/animal	1770

[a] Poly I:C, 1 mg/kg.
[b] Subcutaneous injection.
[c] Units per milliliter of serum.

In order to determine if the injection of drugs actually reduced the interferon level or simply delayed the appearance of interferon in the sera, mice were injected with poly I:C 4 hr after injection of drug and bled at subsequent intervals thereafter rather than the 6–8 hr in the standard procedure. It was found that peak synthesis of interferon by the drug-treated animals was similar to that obtained in control animals, *i.e.*, 4 to 12 hr after poly I:C.

The effects of these drugs on body temperature was considered. Rectal temperatures were measured using a sensitive YSI thermister at various intervals following injection of the narcotics. Results indicated that although

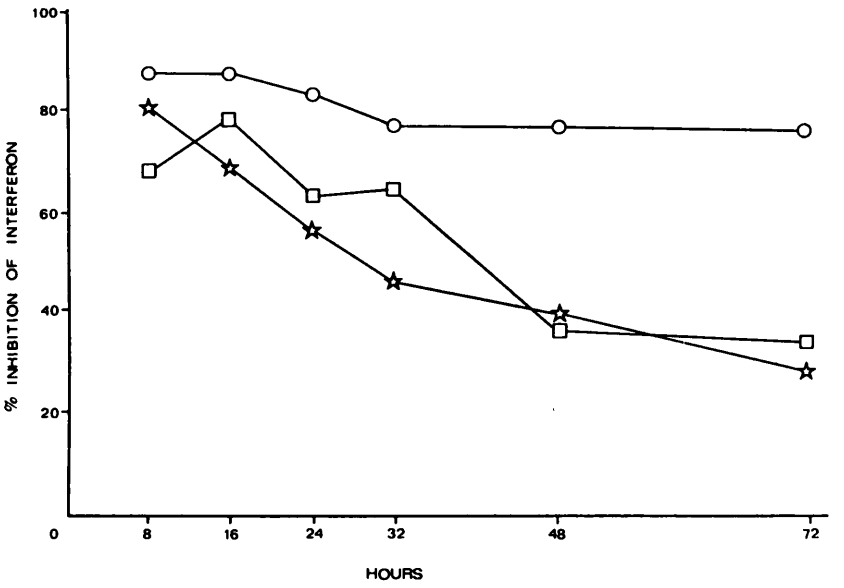

FIG. 2. Effects of narcotic analgesics on the level of circulating interferon. Mice were injected with poly I:C (1 mg/kg) at various intervals after exposure to the drugs. All mice were bled 6 to 8 hr postinjection of poly I:C (★—) morphine, 112.5 mg/kg; (○—) Dilaudid, 50 mg/kg; (□—) methadone, 15.0 mg/kg.

temperature variations did occur between the various treatments and the control, they did not correlate with interferon production.

Tissue levels of interferon were assessed in order to show if they would reflect that found in circulation (sera). In order to demonstrate this, various organs including spleen, liver, lung, and kidney were removed from drug-treated animals, sonified, centrifuged at 1500 rpm/20 min, passed through a 0.22 μm filter and assayed for activity as above. It is clearly shown in Table II that interferon production was also inhibited in the various organs tested. Particularly marked was inhibition in spleen tissue which was only 32% of the control.

The morphine antagonist naloxone was employed to see if it might have an effect on morphine inhibition of interferon production. Although the hyperexcitability induced by morphine was not observed if mice were preinjected with naloxone; naloxone had little effect on the alteration of the morphine effect when administered prior to, with, or post morphine. Naloxone alone caused 60% inhibition of interferon production.

The mechanism of interferon induction by bacterial endotoxin is different from that of poly I:C (4). For this reason, mice were injected with morphine (112.5 mg/kg), 4 hr later a single injection of endotoxin (3.3 mg/kg) was administered either intraperitoneally or intravenously. Mice were bled 2 hr later and the sera were assayed for interferon. The results showed that the interferon titer was higher in mice receiving intravenous

TABLE II. The Effect of Morphine on the Tissue and Circulating Interferon Following Intraperitoneal Injection of Poly I:C.[a]

Sample	Interferon titer[b]	
	Treated[c]	Control[d]
Spleen	1210.7	3748.0
Liver	749.6	1078.0
Lung	568.9	868.7
Kidney	656.2	757.2
Serum	698.8	1957.0

[a] Poly I:C, 1 mg/kg.

[b] Interferon titer (units/100 mg of tissue) or (units/ml of serum).

[c] Morphine, 112.5 mg/kg, sc.

[d] Saline, 0.15 ml/animal, sc.

TABLE III. The Effect of Morphine on the Level of Circulating Interferon in Mice when Endotoxin[a] Was Used as the Inducer.

Route of injection	Treated[b]	Control[c]
Intravenously	236.4[d]	538.5
Intraperitoneally	83.3	123.1

[a] Endotoxin, 3.3 mg/kg.
[b] Morphine, 112.5 mg/kg.
[c] Saline, 0.15 ml/animal.
[d] Interferon titer (units/ml of serum).

injections rather than intraperitoneal injections (Table III). The former was inhibited 56% and the latter 33% by a single injection of morphine.

The previous experiments were concerned with the injection of poly I:C following exposure to drugs. In the next series morphine was injected at various intervals after poly I:C induction. It was found that marked inhibition of interferon production occurred even when poly I:C was injected prior to morphine. When mice were injected with morphine 0.5 hr after poly I:C a 70% reduction of IF was obtained. When the interval was 4 hr a 34% reduction was noted.

In one group of animals single injections of morphine were administered daily for the 10 days prior to injection of poly I:C. Results indicated that multiple injections markedly inhibited the levels of circulating interferon. Interferon titers of sera from mice exposed to either 5 or 10 daily injections of morphine were only about 30% those of the control when injected with poly I:C up to 6 days after the last injection of morphine.

Morphine, Dilaudid, and methadone were tested for their effect on interferon induction by poly I:C *in vitro*. A representative experiment is illustrated by Table IV. In the sequential and combined incubations, the drugs had little effect on the interferon induction system. However, without the interferon inducer the host cells were very susceptible to VSV infection. A second series of experiments were designed to test the effects of drugs on the action of interferon *in vitro*. Cells were incubated with mouse interferon and morphine in various combinations then challenged with VSV. The percentage plaque reduction was the same as in those cultures not exposed to morphine.

Discussion. The precise determination of a minimum dose of drug capable of inhibiting interferon production is extremely difficult. In these studies as little as 1.39 mg/kg morphine was inhibitory to interferon production. This dose did not induce the behavioral response (hyperexcitability, Straub tail) that was obtained with the next higher dose of 4.17 mg/kg. Of considerable importance was the problem of how long after a single injection of drug can one demonstrate an inhibition of poly I:C induction of interferon. It is clear from these studies that the inhibition persisted for at least 9 days.

The mechanism of action of the narcotic analgesics at the cellular level is unknown. However, all had essentially the same effect on interferon synthesis, suggesting a common pathway. Possible factors involved could be: (a) alteration of body temperature, (b) increase of serotonin or other pharmacologically active compounds, and (c) alteration of protein synthesis. A lowering of body temperature is known to decrease interferon synthesis (5). In this study the effect of the drugs on body temperature was minimal with little or essentially no correlation between interferon titer obtained and body temperature. In fact the presence of a reduced capacity for producing interferon 7 and 9 days postinjection or narcotic should effectively rule

TABLE IV. The Effect of Morphine on Interferon Induction *in Vitro*.

	Morphine concn		(μg/ml)
Incubation	500	100	20
Single[a]	5.6[d]	0	7.8
Sequential[b]	100	100	100
Combined[c]	100	100	100
Control[e]	100		

[a] Cells incubated 4 hr with morphine.
[b] Cells incubated 4 hr with morphine, washed 2×, incubated with poly I:C + DEAE (10 and 200 μg/ml) for 2 hr.
[c] Cells incubated 2 hr with morphine and poly I:C + DEAE.
[d] Percentage plaque reduction.
[e] Cells incubated 2 hr with poly I:C + DEAE.

out that the reduction of interferon is mediated through a lowering of body temperature.

Reports in the literature have appeared regarding the presence and/or absence of serotonin levels in brains of animals injected with various narcotic analgesics (6–8). In the present study a preliminary experiment involving the injection of serotonin appeared to have little effect on the induction system, but this alone does not exclude the possibility that other pharmacologically active compounds could play a role in morphine-induced interferon depression. Solomon, Merigan and Levine (9) reported that corticosteroids and ACTH administration did not alter the titers of virus-induced interferon in mice. Hence, the possibility that the drugs inhibit interferon production via the alteration of hormone secretions awaits further experimentation.

Using either single or multiple doses of certain narcotic analgesics Cochin and Axelrod (10) demonstrated that morphine possesses the capability of inhibiting microsomal enzyme synthesis of proteins in the liver. Clouet and Ratner (11) also showed that morphine and other related compounds could affect protein synthesis in a number of other biological systems. It is conceivable that morphine and the other drugs could inhibit interferon production via a general inhibition of protein synthesis.

The morphine antagonist naloxone completely eliminated the behavioral response induced by morphine but did not alter the morphine effect on interferon production. Naloxone alone and in various combinations with morphine was able to inhibit interferon production. This seemed likely in the light of its similarity in structure to morphine.

The mechanism of interferon induction by endotoxin may be different from that of poly I:C. If this is true then morphine was not only capable of inhibiting the synthesis of interferon by poly I:C but also inhibited the release of interferon bound to cellular constituents suggesting a more general effect on poly I:C induction of interferon synthesis.

The daily injections of morphine for a period of 10 days was not sufficient to build up tolerance to morphine as evidenced by the behavioral patterns of treated mice. The effects obtained following daily injections of morphine were similar to those obtained following a single injection. However, the level of inhibition persisted throughout the test period and was maintained at a high level at least 5 days after the last injection.

Results of the *in vitro* studies indicate that the narcotic analgesics had little effect on either interferon induction or action *in vitro*. The action of these drugs on the inhibition of interferon production is still unknown. The characteristics of many of these drugs may be unlike most antibiotics and metabolic inhibitors which are active both *in vivo* and *in vitro*. These drugs may function only in the *in vivo* system possibly through the synthesis of active compounds which in turn cause the inhibition as opposed to direct action at the cellular level. To date no evidence for the presence of these materials has been obtained. Although the effect of morphine on interferon production *in vitro* was not demonstrated clearly, some preliminary experiments suggested that the kinetics of interferon induction and action could be affected slightly by administration of poly I:C immediately prior to the exposure of morphine.

Summary. Morphine, Dilaudid, and methadone were injected into mice in order to assess their effect on interferon production. It was found that all three narcotic analgesics significantly reduced the level of serum interferon induced by either poly I:C or endotoxin. The level of inhibition was directly related to drug dosage. The inhibitory effect of morphine can persist at least 9 days following a single injection. Interferon levels of certain tissues particularly spleen were also depressed in morphine-treated animals. This effect on the level of interferon does not appear to be mediated through an effect on body temperature nor can it be eliminated by the morphine antagonist naloxone. These drugs were also tested on cells *in vitro* using poly I:C as an inducer and do not appear to have a major effect on either the induction or the action of interferon *in vitro*.

The authors thank Dr. John J. Dallman, Department of Community Medicine, Medical College of

Georgia for his assistance in the statistical treatment of these data.

1. Ho, M., *in* "Interferons" (N. B. Finter, ed.), p. 21. Wiley, New York (1967).

2. Vilcek, J., Ng, M. H., Friedman-kien, A. E., and Krawciw, T., J. Virol. **2**, 648 (1968).

3. Wagner, R. R., Levy, A. H., and Smith, T. J., *in* "Methods in Virology" (K. Maramorosch and H. Koprowski, eds.), Vol. 4., p. 1. Academic Press, New York (1968).

4. Stinebring, W. R., and Youngner, J. S., Nature (London) **204**, 712 (1964).

5. Isaacs, A., *in* "Advances in Virus Research" (K. M. Smith and M. A. Lauffer, eds.), Vol. 10, p. 1. Academic Press, New York (1963).

6. Way, E. L., Loh, H. H., and Shen, F., Science **162**, 1290 (1968).

7. Marshall, I., and Grahame-Smith, D. G., Nature (London) **228**, 1206 (1970).

8. Bowers, M. B., and Kleber, H. D., Nature (London) **229**, 134 (1971).

9. Solomon, G. F., Merigan, T. C., and Levine, S., Proc. Soc. Exp. Biol. Med. **126**, 74 (1967).

10. Cochin, J., and Axelrod, J., J. Pharmacol. Exp. Ther. **125**, 105 (1959).

11. Clouet, D. H., and Ratner, M., J. Neurochem. **15**, 17 (1968).

Received Sept. 22, 1972. P.S.E.B.M., 1973, Vol. 142.