Anti-Cancer Drugs 1994, **5**, pp. 90–94

The opioid mechanism of interferon-α action

BT Ho, JG Lu, YY Huo, SH Fan, CA Meyers, LW Tansey,[1] R Payne and VA Levin

Department of Neuro-Oncology, Box 316, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA. Tel: (+1) 713 792 2121; Fax: (+1) 713 794 4999. [1]Department of Nuclear Medicine, Box 59, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd, Houston, TX 77030, USA.

Rats were trained to discriminate the opioid receptor agonist ethylketocyclazocine (EKC) (0.3 mg/kg body weight, intraperitoneally) from saline. Interferon-alpha (IFN-α), when substituted for EKC, elicited a dose-related increase in EKC-like responses. This generalization of EKC responses was blocked by the opioid antagonist naloxone (1 mg/kg). Potentiation of responses to a low dose (0.1 mg/kg) of EKC by IFN-α (1×10^6 U/kg or 0.22 nmol/kg) was also observed. Data thus indicate the involvement of opioid neurons on the action of IFN-α. *d*-Amphetamine (0.8 mg/kg) was shown to potentiate both EKC (0.1 mg/kg) and IFN-α (1×10^6 U/kg). The present study confirms our previously proposed opioid-mediated dopaminergic mechanism of IFN-α.

Key words: Ethylketocyclazocine, *d*-amphetamine, drug discrimination, interferon-α, opioid–dopamine interaction.

Introduction

There is increasing clinical use of biologic response modifiers (i.e. the cytokines) to treat cancer and other diseases. In the treatment of cancer, when high doses or chronic usage of cytokines are required, neurotoxicity often develops to a point that becomes dose limiting. Pronounced symptoms of neurotoxicity include changes in behavior, emotion and cognition; these may be lethargy, hypersomnia, depression, agitation, confusion, memory loss, and problems with verbal abstraction.[1–5] In one population of patients, some affective and cognitive alterations resulting from cytokine therapy persisted for months after the discontinuation of treatment.[5] Quality of life in patients receiving cytokines will be greatly improved if the cytokine-induced neurotoxicity symptoms can be minimized.

This work was supported in part by an institutional PRS Research Grant, NIH–NCI grant CA-16672, Department of Neuro-Oncology Development Fund, and a gift from the Earl A. & Bertha D. Carpenter Research Endowment.

Correspondence to BT Ho

It is postulated that the neurotoxic symptoms are the manifestation of an alteration in neurochemical system(s) that control emotional and/or mental functions. Pharmacologic amelioration of cytokine side effects is possible, if the neurochemical mechanisms of cytokines become understood. In our laboratory, we have selected a 'drug discrimination' technique and used the rodent behavioral model to determine neurochemical systems which are involved in the action of cytokines.

Our previous studies, in rats trained to discriminate *d*-amphetamine from saline, showed that recombinant human interferon-alpha (IFN-α) potentiated responses elicited by *d*-amphetamine.[6] This potentiation of *d*-amphetamine (via dopaminergic mechanism) by the cytokine was suppressed by the opioid antagonist naloxone.[6] The observation of opioid–dopamine (DA) interaction in the behavioral effects of IFN-α was postulated to be a result of the action of IFN-α on central opioid receptors causing the release of presynaptic DA. As a consequence, the cytokine potentiates the discriminative effect of *d*-amphetamine, whose primary neurochemical effects are the release of DA and the blocking of DA re-uptake.[6] To confirm opioid-receptor agonist action of IFN-α, we further trained a group of rats to discriminate an opioid agonist, ethylketocyclazocine (EKC), from saline. In these rats, IFN-α should mimic EKC responses in the discriminative task. Here we report the findings of our study.

Materials and methods

Animals

Male Wistar rats (Charles River Laboratories, Wilmington, MA) that initially weighed 150–200 g were used as subjects. The animals were fed after daily experimental sessions and on weekends in quantities adjusted to maintain the animals at

80–85% of their expected free-feeding weight based on the supplier's growth chart.

Behavioral apparatus

Drug discrimination training was carried out in three two-lever sound-attenuated operant chambers (Coulbourn Instruments, Lehigh Valley, PA). Solid-state programming equipment was used to control the delivery of reinforcement and to record data generated during test and training sessions.

Preliminary training

For 30 min a day, each animal learned to respond with the operant levers for reward (food reinforcement). Reinforcement was given on alternating levers (double-alternating schedule) with delivery of a 45 mg Noyes pellet for initially every five (FR-5), then every 10 (FR-10) and finally every 15 (FR-15) consecutive responses on the correct lever.

Discriminative training and extinction testing

After the animals stabilized under FR-15 schedule, five daily sessions per week of discriminative training began. Fifteen minutes prior to each 30 min training period, each animal was injected intraperitoneally either with (±)-EKC methanesulfonate (0.3 mg/kg) in saline or with saline (1 ml/kg) alone, according to the test day: on the day following EKC administration only responses made on the left lever were reinforced; however, on the day when saline alone was given, reinforcement was contingent on pressing only the right lever. The sequence of weekly 4 day injections of drug and saline was a counterbalance order of all possible combinations with 2 days on the left (drug) lever and 2 days on the right (saline) lever, and no more than two consecutive sessions followed either the training drug (EKC) or saline. EKC was generously supplied by Sterling Research Group (Rensselaer, NY).

To test the accuracy, on day 5 of a week following 4 days of discriminative training, the animals were injected with the training drug or saline 30 min (determined by a time–effect study, Table 1) prior to being placed in the operant chambers with the reinforcement delivery disconnected (extinction). During the 2 min extinction sessions, responses on both levers were cumulatively recorded. The degree of discrimination between the training drug and saline was defined as the percentage of total responses made on the appropriate lever in the absence of reinforcement. Animals were considered accurate when lever responding reached over 80% on the appropriate lever, i.e., after drug administration, at least 80% of the responses were on the drug lever; whereas after saline administration, at least 80% of the responses were on the saline lever. When animals attained over 80% accuracy under both conditions, testing with IFN-α began. Training sessions continued for 4 days a week, with testing on day 5.

Table 1. Production of EKC responses at various times post-injection [a]

Time (min)	EKC lever choice (%)
15	69.2 ± 6.6
30	90.0 ± 3.3
45	75.8 ± 4.9

Each value represents the mean (± SEM) of six animals.
[a] The training dose (0.3 mg/kg) of EKC was administered intraperitoneally to rats trained to discriminate EKC from saline.

Generalization of EKC responses by IFN-α

Extinction sessions were again performed on every fifth day of a week with recombinant human IFN-α (rIFN-α) (generous gift of Dr Michael J. Brunda, Hoffmann-LaRoche, Nutley, NJ). The cytokine (1, 2, 4 and 6 × 10^6 U/kg or 0.22–1.32 nmol/kg) in saline was injected intramuscularly 60 min before the extinction tests. (Our previous study has demonstrated that the optimal IFN-α effect was at 60 min post-injection.[6]) Results were expressed as the number of correct responses divided by the total number of responses.

To examine suppression of IFN-α-induced EKC-like responses, naloxone (0.5 or 1 mg/kg) was injected intraperitoneally together with IFN-α or saline. Naloxone alone did not produce any EKC-like response.[6]

Potentiation of EKC responses by IFN-α

IFN-α (1 or 2 × 10^6 U/kg) was injected intramuscularly 30 min prior to i.p. injection of 0.1 mg/kg EKC (i.e., 60 min before extinction tests). Responses to EKC in the absence and presence of IFN-α were compared. The choice of EKC dose was based on

our finding that this dose produces responses below the maximum of 80–85% on the correct lever (Table 2).

Potentiation of EKC and IFN-α responses by *d*-amphetamine

EKC (0.1 mg/kg) in saline was injected intraperitoneally 15 min prior to 0.8 mg/kg *d*-amphetamine sulfate. IFN-α (1×10^6 U/kg) was administered intramuscularly 45 min before 0.8 mg/kg *d*-amphetamine. Extinction tests were performed 15 min after injection of *d*-amphetamine.

Results

The time–effect study with 0.3 mg/kg (the training dose) of EKC indicates that the maximum effect occurs at 30 min post-injection (Table 1). The dose–response produced at 30 min post-injection in the EKC-trained animals is shown in Table 2.

IFN-α, when substituted for EKC on the test days, elicited a dose-related increase in EKC-like responses in animals trained to discriminate EKC from saline (Table 3). This generalization of EKC responses by IFN-α (4×10^6 U/kg) was suppressed by pretreatment of animals with the opioid antagonist naloxone (Table 4).

Table 2. Production of EKC responses by varying doses of EKC[a]

Dose (mg/kg)	EKC Lever choice (%)
0.05	35.2 ± 4.2
0.1	43.0 ± 8.9
0.2	68.1 ± 6.5
0.3	90.0 ± 3.3
0.4	84.2 ± 4.1

Each value represents mean (± SEM) of six animals.
[a] Respones were measured 30 min after injections of EKC, at the doses shown above, to rats trained to discriminate 0.3 mg/kg EKC from saline.

Table 3. Generalization of responses to EKC by IFN-α

IFN-α (U/kg)	EKC lever choice (%)
1×10^6	52.2 ± 7.8
2×10^6	70.8 ± 6.7
4×10^6	77.6 ± 5.6
6×10^6	83.6 ± 4.8

Mean (± SEM) of six animals.

A potentiation of EKC responses was observed when EKC and IFN-α were co-administered in subthreshold doses that alone did not produce EKC responses (Table 5).

In the EKC-trained animals, a subthreshold dose (0.8 mg/kg) of *d*-amphetamine, which was able to potentiate the low dose EKC (Table 6), was also found to cause potentiation of EKC-like responses induced by IFN-α (Table 7).

Table 4. Supression of IFN-α-induced EKC-like responses by naloxone

Agents (dose)	EKC lever choice (%)
IFN-α (4×10^6 U/kg)	75.2 ± 7.2
Naloxone + IFN-α	
(0.5 mg/kg) (4×10^6 U/kg)	61.5 ± 3.6
(1.0 mg/kg) (4×10^6 U/kg)	38.0 ± 5.7[a]

Each value represents the mean (± SEM) of six animals.
[a] Significantly different from IFN-α(4×10^6 U/kg) control group; $p = 0.0312$ (Wilcoxon test).

Table 5. Potentiation of responses to EKC by IFN-α

Agents (dose)	EKC lever choice (%)
EKC (0.1 mg/kg)	46.2 ± 7.2
IFN-α	
(1×10^6 U/kg)	54.8 ± 8.5
(2×10^6 U/kg)	70.3 ± 8.5
IFN-α + EKC	
(1×10^6 U/kg) (0.1 mg/kg)	71.8 ± 4.0[a]
(2×10^6 U/kg) (0.1 mg/kg)	78.3 ± 8.1[a]

Each value represents the mean (± SEM) of six animals.
[a] Significantly different from EKC (0.1 mg/kg) control group; $p = 0.0312$ (Wilcoxon test).

Table 6. Potentiation of responses to EKC by *d*-amphetamine

Agents (dose)	EKC lever choice (%)
EKC (0.1 mg/kg)	43.5 ± 4.0
d-amphetamine (0.8 mg/kg)	50.6 ± 3.0
d-amphetamine + EKC (0.8 mg/kg) (0.1 mg/kg)	74.8 ± 5.8[a]

Each value represents the mean (± SEM) of six animals.
[a] Significantly different from EKC (0.1 mg/kg) control group; $p = 0.0312$ (Wilcoxon test).

Table 7. Potentiation of IFN-α-induced EKC-like responses to *d*-amphetamine

Agents (dose)	EKC lever choice (%)
IFN-α (1 × 10^6 U/kg)	50.0 ± 6.8
d-amphetamine (0.8 mg/kg)	54.3 ± 8.6
d-amphetamine + IFN-α (0.8 mg/kg) (1 × 10^6 U/kg)	78.8 ± 4.9[a]

Each value represents the mean (± SEM) of six animals.
[a] Significantly different from IFN-α (1 × 10^6 U/kg) control group; $p = 0.0312$ (Wilcoxon test).

Discussion

Data on generalization and potentiation of EKC stimulus by IFN-α indicate that EKC and IFN-α share a common mechanism. Based on our previous studies using rats trained to discriminate *d*-amphetamine from saline, potentiation of *d*-amphetamine responses was postulated to be binding of IFN-α on opioid receptors causing the presynaptic release of DA.[6] The effects of opioid peptides and morphine on DA neurons[7–14] as well as the opioid-like activities of IFN-α are well documented.[15,16] There were reports specifically showing morphine-induced release of DA in the nucleus accumbens and striatum[10–14] as well as blockade of behavioral effects of opioid atgonist by DA receptor antagonists.[17] Perhaps the most direct evidence for the involvement of opioid neurons on the action of IFN-α was our previous finding that both IFN-α and morphine enhanced *d*-amphetamine discrimination in rats trained to discriminate *d*-amphetamine from saline.[6] The present study tends to confirm our proposed neurochemical mechanism of IFN-α.

The action of cytokines on the DA system is not a new concept. For example, using push–pull perfusion technique, interleukin-1 has been shown to stimulate release of DA and its metabolite from the hypothalamus.[18] Our data on the potentiation of both EKC discrimination and the IFN-α induced EKC-like responses by *d*-amphetamine further strengthen an opioid-mediated dopaminergic mechanism of IFN-α.

The present study has illustrated the usefulness of the drug discrimination technique in elucidating the biological effect of IFN-α. It raises the question of which opioid receptor subtypes may be involved. This area of research is currently being pursued in our laboratory. In addition, clinical evaluation of IFN-α-induced neurotoxic symptoms controlled by naltrexone, a long-acting opioid receptor antagonist, is under way.

References

1. Adam F, Quesada JR, Gutterman JU. Neuropsychiatric manifestations of human leukocyte interferon therapy in patients with cancer. *JAMA* 1984; **252**: 938–41.
2. Denicoff KD, Rubinow DR, Papa MZ, *et al.* The neuropsychiatric effects of treatment with interleukin-2 and lymphokine-activated killer cells. *Ann Intern Med* 1987; **107**: 293–300.
3. Renault PF, Hoofnagle JH, Park Y, *et al.* Psychiatric complications of long-term interferon alpha therapy. *Arch Intern Med* 1987; **147**: 1577–80.
4. Meyers CA, Obbens EAMT, Scheibel RS, *et al.* Neurotoxicity of intraventricularly administered alpha-interferon for leptomeningeal disease. *Cancer* 1991; **68**: 88–92.
5. Meyers CA, Scheibel RS, Forman AD. Persistent neurotoxicity of systemically administered interferon-α. *Neurology* 1991; **41**: 672–6.
6. Ho BT, Huo Y-Y, Lu J-G, *et al.* Opioid–dopamine mechanisms in the potentiation of *d*-amphetamine discrimination by interferon-α. *Pharmacol Biochem Behav* 1992; **42**: 57–60.
7. Chiara D, Imperato A. Opposite effects of mu- and kappa-opiate agonists on dopamine-release in the nucleus accumbens and in the dorsal caudate of freely-moving rats. *J Pharmacol Exp Ther* 1988; **244**: 1067–80.
8. Gysling K, Wang RY. Morphine-induced activation of A10 dopamine neurons in the rats. *Brain Res* 1983; **277**: 119–27.
9. Matthews RT, German DC. Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine. *Neuroscience* 1984; **11**: 617–25.
10. Jackson D, Enden NE, Dahlstrom A. A functional effect of dopamine in the nucleus accumbens and in some other dopamine-rich parts of the rat brain. *Psychopharmacologia* 1975; **45**: 139–49.
11. Lal S, Sourkes TL, Missola K, *et al.* Effect of apomorphine and emetine alkaloids on central dopa-minergic mechanisms in rats. *Eur J Pharmacol* 1972; **20**: 71–9.
12. Pijnenburg AJ, Honig W, van Rossum J. Antagonism of apomorphine and *d*-amphetamine induced stereotyped behavior by injection of low doses of haloperidol into caudate nucleus and the nucleus accumbens. *Psychopharmacologia* 1975; **45**: 65–71.
13. Pijnenburg AJ, Honig W, *et al.* van Rossum J. Effects of chemical stimulation of the mesolimbic dopamine system upon locomotor activity. *Eur J Pharmacol* 1976; **35**: 45–58.
14. Weissman A. Cliff jumping in rats after intraventricular treatment with apomorphine. *Psychopharmacologia* 1971; **21**: 60–5.
15. Dafny N, Prieto-Gomez B, Reyes-Valzquez C. Does the immune system communicate with the central nervous

system? Interferon modifies central nervous activity. *J Neuroimmunol* 1985; **9**: 1–12.

16. Nakashima T, Hori T, Kuriyama K, *et al.* Naloxone blocks the interferon-α induced changes in hypothalamic neuronal activity. *Neurosci Lett* 1987; **82**: 332–6.
17. Morelli M, Fenu S, Chiara GD. Substantia nigra as a site of origin of dopamine-dependent motor syndromes induced by stimulation of mu and delta opioid receptors. *Brain Res* 1989; **487**: 120–30.
18. Mohankumar PS, Thyagarajan S, Quadri SK. Interleukin-1 stimulates the release of dopamine and dihydroxyphenylacetic acid from the hypothalamus *in vivo*. *Life Sci* 1991; **48**: 925–30.

(Received 27 September 1993; received in revised form 20 October 1993; accepted 21 October 1993)