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Opioid–Dopaminergic Mechanisms in the Potentiation of *d*-Amphetamine Discrimination by Interferon-α

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HO, B. T., Y.-Y. HUO, J.-G. LU, L. W. TANSEY AND V. A. LEVIN. *Opioid-dopaminergic mechanisms in the potentiation of* d*-amphetamine discrimination by interferon-α.* PHARMACOL BIOCHEM BEHAV **42**(1) 57–60, 1992.— In rats trained to discriminate 0.8 mg/kg IP *d*-amphetamine from 1 ml/kg saline, 4 × 10^6 U/kg of recombinant human interferon-α (rIFN-α) given intramuscularly 1 h prior to tests potentiated responses elicited by 0.4 mg/kg *d*-amphetamine. Coadministration of the opioid receptor antagonist naloxone (1 mg/kg IP) with rIFN-α suppressed the potentiation of *d*-amphetamine by the cytokine. Opioid-dopaminergic mechanisms are proposed to explain the action of rIFN-α.

Interferon-α   *d*-Amphetamine   Naloxone   Opioid   Dopamine   Discriminative responding

IN approximately 30% of cancer patients who receive prolonged and high doses of the cytokine interferon-α, extrapyramidal (Parkinson-like) symptoms featuring rigidity and tremor (19,20) occur, implying the involvement of dopamine (DA) system in the cytokine's action. In one population of patients, the neurotoxic side effects of IFN-α therapy persisted long after treatment was discontinued (20). Our laboratory has been engaged in the utilization of the drug discrimination method for studying neurochemical mechanisms of centrally active drugs. In particular, in rats trained to discriminate *d*-amphetamine from saline their responses to *d*-amphetamine is mediated exclusively by DA (7). We have, therefore, chosen this behavioral paradigm to test the role of DA in the action of IFN-α. Also, because of reports that indicate the opioid-like activities of IFN-α (3,4,23), morphine and naloxone were both used as indicators of opioid involvement in the IFN-α-induced enhancement of *d*-amphetamine discriminative stimulus. The present study demonstrates an opioid–dopamine interaction in the effect of IFN-α on *d*-amphetamine discrimination.

METHOD

Animals

Eighteen male Wistar rats (Charles River Laboratories Inc., Wilmington, MA) that initially weighed 150–200 g were used as subjects. 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-level sound-attenuated operant chambers (Coulbourn Instruments, Inc., Lehigh Valley, PA). Solid-state programming equipment was used to control the delivery of reinforcement and 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 5 (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 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

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TABLE 1

POTENTIATION OF RESPONSES TO *d*-AMPHETAMINE (VIA DA) BY IFN-α

Agents	Dose	Amphetamine Lever Choice (%)		
		1 h*	2 h†	4 h†
d-Amphetamine	0.4 mg/kg	65.3 ± 5.1	55.8 ± 9.7	69.7 ± 8.0
IFN-α	4 × 10^6 U/kg			
+ *d*-Amphetamine	0.4 mg/kg	89.0 ± 2.9‡	58.5 ± 7.5§	69.3 ± 7.5¶
d-amphetamine	0.4 mg/kg	65.2 ± 9.6	–#	–#
IFN-α	1 × 10^6 U/kg			
+ *d*-Amphetamine	0.4 mg/kg	79.2 ± 7.6¶	–#	–#

Each value represents the mean (± SE) of *nine or †six animals.
‡p = 0.004; §p = 1.00; ¶p = 0.563 (Wilcoxon test).
#Not tested.

injected IP either with *d*-amphetamine sulfate (0.8 mg/kg) (11) in saline or with saline (1 ml/kg) alone according to the test day: On the day following amphetamine 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 (amphetamine) or saline.

To test accuracy, on the fifth day of a week following 4 days of discriminative training animals were injected with the training drug or saline 15 min 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, that is, 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 the fifth day.

Potentiation of d-*Amphetamine 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 of Hoffmann-La Roche, Inc., Nutley, NJ). The cytokine (1 and 4 × 10^6 U/kg or 0.22 and 0.89 nmol/kg) in saline was injected IM 45, 105, or 225 min prior to IP injection of 0.4 mg/kg *d*-amphetamine (i.e., 1, 2, or 4 h before extinction tests). Results were expressed as the number of correct responses divided by the total numbers of responses. Responses to *d*-amphetamine in the absence and presence of IFN-α were compared. The choice of amphetamine dose was based on our previous finding that this dose produces responses below the maximum of 80–85% on the correct lever (7–9).

To examine suppression of IFN-α-induced potentiation of *d*-amphetamine discrimination, naloxone (1.0 mg/kg) was injected, IP, together with IFN-α or saline prior to *d*-amphetamine. In another experiment, the same dose of naloxone was given with *d*-amphetamine instead of with IFN-α.

Potentiation of d-*Amphetamine Responses by Morphine*

Morphine sulfate (2 and 3 mg/kg) in saline was injected IP 30 min prior to 0.4 mg/kg *d*-amphetamine sulfate. Extinction tests were performed 15 min after administration of *d*-amphetamine.

RESULTS

In animals trained to discriminate 0.8 mg/kg *d*-amphetamine from saline, decreasing amphetamine dose to 0.4 mg/kg reduced the percentage of responses on the amphetamine lever below the maximum 80% level [Table 1 and (7)]. However, pretreatment with 4 × 10^6 U/kg IFN-α 45 min prior to 0.4 mg/kg *d*-amphetamine, that is, 1 h before extinction tests, increased responses on the amphetamine lever back to the maximum level. IFN-α's potentiation of *d*-amphetamine responding was tested at three different time intervals. The po-

TABLE 2

NALOXONE SUPPRESSION OF IFN-α-INDUCED POTENTIATION OF *d*-AMPHETAMINE DISCRIMINATION

Agent(s)	Dose	Amphetamine Lever Choice (%)
IFN-α	4 × 10^6 U/kg	74.0 ± 6.0*
+*d*-Amphetamine	0.4 mg/kg	
Naloxone‡	1 mg/kg	47.2 ± 8.2*¶
+IFN-α	4 × 10^6 U/kg	
+*d*-Amphetamine	0.4 mg/kg	
IFN-α	4 × 10^6 U/kg	78.6 ± 6.5†
+*d*-Amphetamine	0.4 mg/kg	
Naloxone§	1 mg/kg	62.6 ± 11.0†#
+IFN-α	4 × 10^6 U/kg	
+*d*-Amphetamine	0.4 mg/kg	

Each value represents the mean (± SE) of *nine animals or †six animals.
‡Naloxone was administered with IFN-α.
§Naloxone was administered with *d*-amphetamine.
¶p = 0.004; #p = 0.313 (Wilcoxon test).

TABLE 3
EFFECT OF NALOXONE ON
d-AMPHETAMINE RESPONDING

Agents	Dose	Amphetamine Lever Choice (%)
d-Amphetamine	0.4 mg/kg	61.0 ± 6.4
Naloxone*	1 mg/kg	56.6 ± 9.9†
+ *d*-Amphetamine	0.4 mg/kg	

Each value represents the mean (± SE) of five animals.
*Naloxone was administered 45 min prior to *d*-amphetamine (i.e., 1 h before test).
†$p = 0.563$ (Wilcoxon test).

tentiation was only observed when IFN-α was injected 1 h before tests (i.e., 45 min prior to *d*-amphetamine) (Table 1). The effect of IFN-α was not observed when the cytokine was administered either 2 or 4 h before tests (Table 1). A reduced dose (1×10^6 U/kg) of IFN-α failed to produce significant potentiation of *d*-amphetamine discrimination (Table 1).

When the opioid antagonist naloxone was coadministered with IFN-α, it significantly suppressed the potentiation of *d*-amphetamine responding produced by the cytokine (Table 2). Naloxone alone was found to have no effect on the responses produced by 0.4 mg/kg *d*-amphetamine (Table 3). Timing of naloxone administration was important: Naloxone did not suppress potentiation when given with *d*-amphetamine (i.e., 15 min before tests) instead of with IFN-α (i.e., 1 h before tests) (Table 2).

Potentiation of animals' response to 0.4 mg/kg *d*-amphetamine was also achieved by both 2 and 3 mg/kg morphine (Table 4).

DISCUSSION

This study shows that IFN-α can enhance discrimination between *d*-amphetamine and saline. This *d*-amphetamine-induced discriminative effect is mediated via a dopaminergic mechanism (7), a conclusion based on the finding that DA receptor blockers such as pimozide and haloperidol block the amphetamine-like responses in animals trained to discriminate *d*-amphetamine from saline (2,7,10,28). Neurochemical specificity of amphetamine discrimination was established by the failure of receptor antagonists of neurotransmitter systems other than the DA system to modify the *d*-amphetamine lever responding (7). Because the blockade of *d*-amphetamine stimulus by α-methyl-*p*-tyrosine (7,14) is associated with a selective depletion of newly synthesized catecholamines (32), *d*-amphetamine discriminative effect likely depends on the availability of the store of DA. The results of this study indicate, therefore, the involvement of a dopaminergic mechanism in IFN-α's action.

Evidence for a central mediation of the discriminative stimulus effect of *d*-amphetamine has been demonstrated by experiments in which centrally administered *d*-amphetamine (via lateral ventricular cannulae) generalized to the systemic *d*-amphetamine training condition (27,29) and *p*-hydroxyamphetamine, a polar metabolite of amphetamine, failed to produce generalization to *d*-amphetamine (13). *p*-Hydroxyamphetamine, although equally as active as amphetamine peripherally, is virtually devoid of central stimulant properties because of its high polarity and consequently poor penetration into the CNS.

Based on the above, our finding of the potentiation of *d*-amphetamine discriminative effect by systemically administered IFN-α indicates that the action of the cytokine is on the central dopaminergic system. Our observation that naloxone blocked the potentiating action of IFN-α on *d*-amphetamine responses further implies involvement of the opioid neurons in the regulation of IFN-α action on dopaminergic function. The action of naloxone appears to be time dependent as the suppression of IFN-α-induced potentiation was not observed when naloxone was coadministered with *d*-amphetamine instead of given with IFN-α.

Influence of opioid peptides on midbrain dopaminergic neurons is well documented. Endogenous opioid peptides coexist with DA cell bodies of nigrostriatal and mesolimbic/mesocortical systems, and opioid receptors have been found on DA neurons (16). Furthermore, experimental findings show that morphine causes excitation of DA neurons (6,17). Opioid receptors are involved in the presynaptic release of DA (1). A low dose of morphine elicits central effects by stimulation of DA synthesis, as well as by a parallel increase in the release of newly synthesized DA in the nucleus accumbens and striatum (12,15,24,25,31). More specifically, the behavioral effects of opioid mu- and delta-agonists can be blocked by both D_1 and D_2 receptor antagonists (22).

IFN-α has been found to possess opioid-like activities that include alteration of single-cell activity in hypothalamic and hippocampal nuclei (3). This IFN-α-induced change in neuronal activity is reversed by naloxone (23). Also, morphine withdrawal to naloxone is prevented by IFN-α (3). Taking into account all the above evidence, which clearly indicates the involvement of opioid receptors in presynaptic DA release, and the opioid-like activities of IFN-α, it is tempting to speculate that IFN-α acts on central opioid receptors, causing the release of DA. As a consequence, IFN-α potentiates the discriminative effect of *d*-amphetamine, whose primary neurochemical actions are the release of DA and the blocking of DA reuptake (5,18,26,30,32). This proposed opioid-receptor agonist action of IFN-α is further confirmed by our finding that morphine can be substituted for IFN-α in the potentiation of *d*-amphetamine responses. Analogous to IFN-α's action on the DA system is the recent report in which push-pull perfusion of another cytokine, interleukin-1, caused a stimulation of release of DA and its metabolite 3,4-dihydroxyphenylacetic acid from the hypothalamus (21).

The enhancement of *d*-amphetamine by IFN-α may not be related to the production of Parkinson-like symptoms in patients under IFN-α medication. Nevertheless, our present

TABLE 4
POTENTIATION OF RESPONSES TO
d-AMPHETAMINE BY MORPHINE

Agents	Dose	Amphetamine Lever Choice (%)
d-Amphetamine	0.4 mg/kg	54.3 ± 5.8
Morphine	2 mg/kg	72.3 ± 4.9*
+ *d*-Amphetamine	0.4 mg/kg	
d-Amphetamine	0.4 mg/kg	50.6 ± 3.0
Morphine	3 mg/kg	79.8 ± 3.8†
+ *d*-Amphetamine	0.4 mg/kg	

Each value represents the mean (± SE) of eight animals.
*$p = 0.014$; †$p = 0.008$ (Wilcoxon test).

study indicates involvement of opioid–dopamine interaction in the biological effect of IFN-α. In our laboratory, we are currently studying this interaction at pre- and postsynaptic levels.

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