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INFLUENCE OF NALOXONE ON THE EFFECTS OF LSD IN MONKEYS

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Summary—Three stump-tailed monkeys were trained in an appetitive behavioral task. Administration of 0.1 mg/kg of lysergic acid diethylamide (LSD) moderately disrupted performance acutely, and produced abnormal motor activity. With repeated administration of LSD, motor activity and task responding returned to normal.

When 1.0 mg/kg of naloxone was administered prior to administration of LSD in a crossover design, appetitive responding was abolished acutely in all animals and behavior was substantially more disrupted than with LSD alone. Tolerance did not occur with repeated administration of this combination of drugs. Endorphins and/or opiate receptors may act to attenuate the effects of hallucinogenic agents, and may subserve the development of tolerance to these effects.

Key words: LSD, hallucinogens, tolerance, opiates, primates.

Compounds with hallucinogenic activity, including *N*,*N*-dimethyltryptamine (DMT), occur naturally in the brain (Wyatt, Mandel, Ahn, Walker and Vanden Heuvel, 1973; Wyatt, Termini and Davis, 1971; Saavedra and Axelrod, 1972) and may be related to the development of some forms of schizophrenia (Osmond and Smythies, 1952; Barchas, Elliott and Berger, 1977). Endorphins and opiate receptors in brain may also be involved in the disease process (Berger, 1978; van Ree and DeWied, 1981).

Recently, opiates have been observed to have significant behavioral interactions with hallucinogens in rats. Morphine and methadone, in appropriate doses, significantly antagonized the disruption of bar-pressing behavior caused by DMT or LSD (Ruffing and Domino, 1981). Conversely, pretreatment with naloxone has been shown to significantly prolong catalepsy induced by LSD in rats (Ruffing and Domino, 1981) and to potentiate the disruption of food-rewarded bar-pressing behavior induced by DMT, LSD or mescaline (Fertziger and Fischer, 1977; Ruffing, Kovacic, Demetriou and Domino, 1979; Commissaris, Moore and Rech, 1980).

The goal of this study was to determine whether naloxone alters the acute effects of LSD in primates and/or the development of tolerance to these effects.

METHODS

Three stump-tailed monkeys (*Macaca arctoides*), weighing 3.5, 3.7 and 7 kg, were trained in a food-rewarded task. During training and testing the animals were moved to a separate room and placed in a cage identical to their home cage except that the front bars were replaced with clear plexiglass.

The monkeys were trained to reach through a diamond-shaped hole in the center of the plexiglass sheet in order to pluck a piece of marshmallow from a wire as it revolved outside the cage in a one-meter circle parallel to the floor. The marshmallow was within reach for approx. 0.5 sec during each 3-sec revolution. After each reward a mandatory 15-sec "reloading" time was imposed.

All monkeys mastered this task easily in one training session and were consistent in their performance in control testing sessions both within and among individuals. Subjects were tested twice each day for the remainder of the study, with approx. 10 hr elapsing between the morning and afternoon sessions (36 consecutive test sessions).

The animals were injected with 0.05 ml/kg of vehicle or solution of drug into a thigh muscle, 30 and 15 min prior to the start of each testing session. The investigator administering the solutions and testing the animals was "blind" with respect to the identity of the solutions. The number of food rewards obtained and the general behavior were recorded during each 5-min test session.

(+)-Lysergic acid diethylamide (+)-tartrate (supplied by National Institute on Drug Abuse) was combined with 9 parts of USP dextrose in a mortar and pestle to facilitate accurate weighing and delivery. Naloxone HCl was obtained from Endo Laboratories. Injections of vehicle consisted of 0.1 M dextrose solution. Data were analyzed with an Apple IIe computer, using a standard program for analysis of variance for repeated measures with equal number. The order of injection is summarized in Table 1.

RESULTS

Lysergic acid diethylamide (LSD; 0.1 mg/kg) moderately disrupted the performance on the appetitive task initially, but became progressively less disruptive

Table 1. Experimental design

Sessions	Injected 30 min prior to each test	Injected 15 min prior to each test
1–8	Vehicle	Vehicle
9–16	Vehicle	0.1 mg/kg LSD
17–23	Vehicle	Vehicle
24–31	1.0 mg/kg Naloxone	0.1 mg/kg LSD
32–34	1.0 mg/kg Naloxone	Vehicle
35–36	Vehicle	Vehicle

Each subject participated in two sessions per day.

with repeated administration (Fig. 1). Abnormal motor effects consisted primarily of ataxic gait and posture, with repetitive twitching or jerking movements of the extremities and head. After 6 to 8 sessions all animals returned to baseline levels of task performance and appeared to have normal motor activity. With subsequent injections of vehicle the subjects continued their previous stable level of responding.

Pretreatment with naloxone (1.0 mg/kg) followed by the administration of 0.1 mg/kg LSD acutely abolished responding in all animals. Jerking movements and ataxia were substantially more pronounced than with administration of LSD alone. In addition, other behavioral abnormalities were observed which had occurred in pilot studies only at dose levels of 1–2 mg/kg LSD. These included periods of lying prone on the cage floor, grasping and clinging to the side of the cage, prolonged glassy staring into space, and episodes of biting the hands and feet.

Tolerance did not develop to these behavioral effects when naloxone was administered with LSD; rather they became more pronounced. Although the response rates for the task increased slightly after the first session, responding became progressively depressed in subsequent sessions. The naloxone–LSD regimen was discontinued when it became apparent that the animals were unable to respond and when concern developed about their well-being.

When naloxone was then administered with vehicle, the animals appeared normal behaviorally and responsiveness on the task resumed. The observation that the small dose of naloxone alone did not adversely affect the behavior is compatible with other reports (Goldberg, Morse and Goldberg, 1981; Spealman, Kelleher, Morse and Goldberg, 1981). The final two vehicle-only sessions produced normal performance and behavior.

DISCUSSION

Endogenous hallucinogens have been implicated in the etiology of schizophrenia (Osmond and Smythies, 1952; Barchas *et al.*, 1977). *N,N*-Dimethyltryptamine (DMT), a potent hallucinogenic agent in man, and other substances (3,4-dimethoxyphenylalanine [DMPEA] and bufotenine), which may have similar properties, have been identified by mass spectrometry and other means in the brain, blood and urine of both normals and schizophrenics (Wyatt *et al.*, 1971, 1973; Saavedra and Axelrod, 1972). Some, but not all, of these studies have demonstrated higher levels in schizophrenics. Other hallucinogens, such as mescaline and psilocin, bear close structural resemblance to neurotransmitters (norepinephrine and serotonin, respectively) and may be produced by faulty enzymatic methylation (Wyatt, Saavedra and Axelrod, 1973; Nichols, Wyatt and Pollin, 1974).

Although unique or elevated levels of hallucinogenic compounds have not been reliably identified in schizophrenics, such substances may be tightly bound to tissue receptors, rapidly degraded, or undetectable for other reasons in body fluids. Alternatively, a critical difference may exist in the receptors themselves, or in the kinetics of the interaction between agonist and receptor.

An objection (Abramson, Jarvik, Gorin and Hirsch, 1956) to the endogenous hallucinogen hypothesis has been that, with frequently repeated administration, a tolerance develops to hallucinogenic agents, as manifested by their inability to continue to produce psychological effects in man and behavioral effects in animals (Appel and Freedman, 1968; Freedman, Appel, Hartman and Molliver, 1964). How then could such substances account for the protracted, often deteriorating, course of schizophrenia? This objection may be answered in part by studies which demonstrate that, in certain paradigms, tolerance does not develop to the effects of repeated administration of a hallucinogen (Hadorn, Mandell and Segal, 1976; Bridger, Mandel and Stoff, 1973).

The discovery of enkephalins and endorphins, polypeptides in brain which bind to opiate receptors

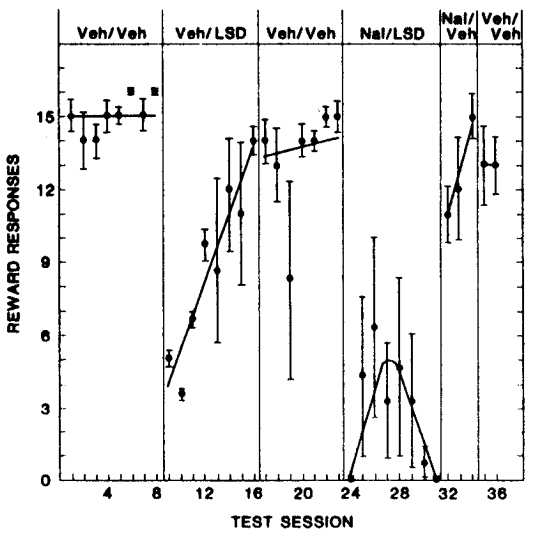

Fig. 1. Effects of several combinations of intramuscular injections of vehicle (Veh), lysergic acid diethylamide (LSD; 0.1 mg/kg) and naloxone (Nal; 1 mg/kg) on an appetitive task in monkeys ($n = 3$). Test sessions were twice daily for 18 consecutive days. Values are shown are means + SE. Analysis of variance for repeated measures revealed significant differences between treatments (F ratio 34.5) with $P < 0.01$ for Veh/LSD vs Nal/LSD by the Newman–Keuls t-statistic.

and have opiate-like activity, provided a new dimension to the study of schizophrenia. While the administration of these substances or of naloxone to schizophrenics has produced variable results, there is evidence demonstrating improvement of symptoms following the administration of endorphins (Verhoeven, van Pragg, van Ree and DeWied, 1979; Jorgansson, Fog and Veilis, 1979). Similarly, morphine and other opiates have elicited favorable results in the treatment of schizophrenia (Comfort, 1977; Gold, Donabedian, Dillard, Slohetz, Riordan and Kleber, 1977), although these agents have been supplanted for this purpose by the neuroleptics. An imbalance or abnormality in endorphin type, level or activity is currently believed to be related to the schizophrenic process (van Ree and DeWied, 1981).

The results of this experiment confirm and extend observations made in studies in rats (Ruffing and Domino, 1981; Fertziger and Fischer, 1977; Ruffing *et al.*, 1979; Commissaris *et al.*, 1980), indicating that naloxone exacerbates the adverse behavioral effects of LSD. It appeared to the present authors, using behavioral and performance criteria, that naloxone increased by about 10-fold the effective potency of LSD. Naloxone did not, however, appear to increase significantly the duration of action of LSD. Subjects receiving naloxone and LSD became behaviorally normal and accepted food by hand within 2 hr after an injection of LSD, and, this was comparable to what was observed during the sessions with LSD alone. In addition, behavior and performance in session 33 (first naloxone–vehicle session after the naloxone–LSD series) were essentially normal, ruling out prolonged action of the naloxone–LSD combination.

Concomitant administration of naloxone prevented the development of tolerance to the disruptive actions of LSD. As far as is known, this is the first demonstration of failure of the development of tolerance to the effects of LSD.

These results, along with those of previous studies, suggest that endorphins and/or opiate receptors attenuate the severity and persistence of the effects of exogenous hallucinogens. It may be that endorphins and exogenous hallucinogens interact within a shared receptor complex, the endorphin component muting the effect of the hallucinogen and subserving the development of tolerance. In support of this concept, tolerance to exogenous hallucinogens apparently represents a change in the activity of receptors, as concentrations of hallucinogens in blood and brain of tolerant animals are not significantly different from those in non-tolerant animals (Winter, 1971).

Presumably, these interactions relate to endogenous hallucinogens as well. The effects of endogenous hallucinogens may normally be attenuated through interaction with endorphins or their receptors. Brains of schizophrenics are markedly deficient in opiate receptors (Reisine, Rossor, Spokes, Iverson and Yamamura, 1980), and this could permit endogenous hallucinogens to produce significant, sustained disturbances in mental function.

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