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Interaction of Synthetic Opioid Metenkephalin Peptide Analogs, Lilly 127623 and FK 33-824 with Indole Hallucinogens: Antagonism of N,N-Dimethyltryptamine- and LSD-Induced Disruption of Food-Rewarded Bar Pressing Behavior in the Rat

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Abstract. The selected opioid metenkephalin synthetic peptide analogs Lilly (LY) 127623 and FK 33-824 were tested for behavioral dose effects and potential interaction with N,N-dimethyltryptamine (DMT) and lysergic acid diethylamide-25 (LSD) in adult male Holtzman rats trained on a positive reinforcement fixed-ratio 4 (FR-4) behavioral bar pressing schedule, i.e., a reward of 0.01 ml sugar-sweetened evaporated milk was earned on every fourth bar press. DMT (3.2 mg/kg) and LSD (0.1 mg/kg), administered IP following a 0.9% NaCl 15–20-min control pretreatment, disrupted established food-rewarded FR-4 bar pressing in a consistent and reproducible manner. Animals pretreated IP with predetermined behaviorally noneffective doses of LY 127623 (0.01–0.32 mg/kg) and FK 33-824 (0.001–0.01 mg/kg) 15–20 min prior to receiving DMT demonstrated significant antagonism to DMT-induced disruption of FR-4 bar pressing, while doses of 0.10–0.32 mg/kg LY 127623 and 0.00032–0.0032 mg/kg FK 33-824 significantly antagonized LSD-induced behavioral effects.

Key words: Opioids – Metenkephalin – Peptides – LY 127623 – FK 33-824 – DMT – LSD – Antagonism – Operant behavior

A therapeutic or pathogenic involvement of opioids in psychoses has been indicated by numerous biological and pharmacological investigations (Barchas et al. 1980; van Praag and Verhoeven 1980; deWied 1980; Marx 1981; Anokhina 1982). These studies, coupled with the recent evidence of multiple opiate receptors and endogenous opioid-like peptides, have stimulated interest in the potential behavioral and therapeutic applications of endogenous endorphins, enkephalins, and their related synthetic analogs (Gesellchen and Zimmermann 1981; Martin 1981; Van Ree and deWied 1981; Szara 1982).

In recent investigations we reported that the opioid antagonists naloxone and naltrexone potentiated induced disruptive effects of N,N-dimethyltryptamine (DMT) and LSD on food-rewarded fixed-ratio (FR-4) behavior in the rat, whereas low doses of the opioid agonists morphine and methadone antagonized the behavioral effects of DMT and LSD, while larger doses selectively potentiated only the effects of LSD (Ruffing and Domino 1979, 1981). The purpose of the present study was to determine possible interactions of selected metenkephalin synthetic peptide analogs with DMT and LSD using rodents in an operant FR-4 behavioral paradigm.

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Materials and Methods

Adult male Holtzman rats (at least 90 days of age) were housed separately in a constant temperature- and humidity-controlled environment. Animals were maintained at approximately 70% of their expected free-feeding weight.

Procedures. The methods for establishing stable food-rewarded FR-4 bar pressing and experimental design parameters have been described in detail (Ruffing and Domino 1981). In brief, rats were trained to bar press daily for sessions of 60-min duration on a FR schedule to receive a reward of 0.01 ml sweetened evaporated milk solution as positive reinforcement on every fourth bar press. Operant behavioral experiments were performed in an isolated, darkened room using Lehigh Valley Electronics rodent operant test chambers (model 143-21) and Gerbrand cumulative recorders.

Subsequent to established, stable FR-4 bar pressing, rats were randomly scheduled to assigned drug and dose groups ($N = 6–12$) and daily bar pressing sessions of 60-min duration for at least 5 days prior to injections. Each animal served as its own control to compare hallucinogen-induced effects with and without assigned pretreatment drugs, with an intervening minimum 7-day drug-free interval between scheduled injections. Prior to the assigned hallucinogen injection (3.2 mg/kg DMT or 0.1 mg/kg LSD IP), control groups were pretreated (15–20 min) with 0.9% NaCl (IP), whereas drug-pretreated groups received a predetermined behaviorally noneffective dose of LY 127623 (0.0032–0.32 mg/kg IP) or FK 33-824 (0.0001–0.01 mg/kg IP) as pretreatment 15–20 min prior to DMT or LSD. The pretreatment drug doses described demonstrated no effect on bar pressing for 120-min periods, as predetermined in pilot studies (Domino and Ruffing 1982). Drugs were administered IP and dosages refer to free base content.

Data Analysis. The duration of drug effect was determined from the length of the horizontal line generated by the cumulative recorder illustrating the onset, duration, and cessation of drug effects or disruption of bar pressing behavior. Two-tailed Student's *t*-test paired comparisons, calculated according to Snedecor and Cochran (1967), were used to analyze the statistical significance of drug effects for control and corresponding drug-pretreated groups, i.e., antagonism, potentiation, or no effect on the behavioral FR-4

disruptive effects characteristically induced by DMT and LSD.

Drugs. DMT was obtained from Sigma (St. Louis, MO, USA) and solutions were prepared by dissolving the free base in 1 N HCl, adding 0.9% NaCl, and titrating the acidity with 0.1 N NaOH to adjust pH to 4.5–5.0. LSD-25 (Delysid) was obtained from the National Institute on Drug Abuse and was administered in 0.9% NaCl. Lilly (LY) 127623 (Metkephamid) and FK 33-824 log doses were administered in 0.9% NaCl, and supplied courtesy of Dr. R. Frederickson (Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN, USA) and Drs. Graffenried and del Pozo (Department of Experimental Therapeutics, Biololgical and Medical Research Division, Sandoz Limited, Basel, Switzerland), respectively.

Results

Characteristic gross behavioral effects of DMT and LSD were evident 2–5 min following IP injections and consisted of lying quietly with eyes open in an excessively flattened position for prolonged intervals, periodic display of Straub tail with or without locomotor or body movements, circling or reverse crawling in a flattened position, splayed legs or extension of the hind limbs, frequent stereotypic and repetitive head or body movements, sustained periods of arching, and disinterest in food or bar pressing. The gross behavioral effects of DMT (3.2 mg/kg) and LSD (0.1 mg/kg) were reproducible and commensurable with the duration of induced disruption of food-rewarded FR-4 bar pressing behavior.

In the LY 127623 and FK 33-824 pretreatment groups, gross behavioral effects following DMT or LSD were usually minimal or absent, although atypical gross behavioral effects were noted in several animals ($N = 4$), i.e., mild to moderate increase in behavioral locomotor activity involving excessive exploring, grooming, and periodic interest in the bar, which may or may not have resulted in pressing for food reinforcement. Following the cessation of drug effects, rats would appear normal, groom briefly, and abruptly start and continue bar pressing at preinjection response rates. Typically, cumulative bar pressing records of rats trained on food-rewarded FR-4 schedules show constant high rates of response with momentary postreinforcement pausing (Ferster and Skinner 1957; Wenger 1980).

The synthetic metenkephalin peptide pretreatment groups showed that some doses of LY 127623 (0.01–0.32 mg/kg) and FK 33-824 (0.00032–0.01 mg/kg) significantly antagonized the disruption of FR-4 bar pressing induced by DMT and LSD. As illustrated in Fig. 1, pretreatment doses of LY 127623 (0.01–0.32 mg/kg) and FK 33-824 (0.001–0.01 mg/kg) significantly antagonized DMT-induced disruption of FR-4 bar pressing, while significant antagonism to the LSD-

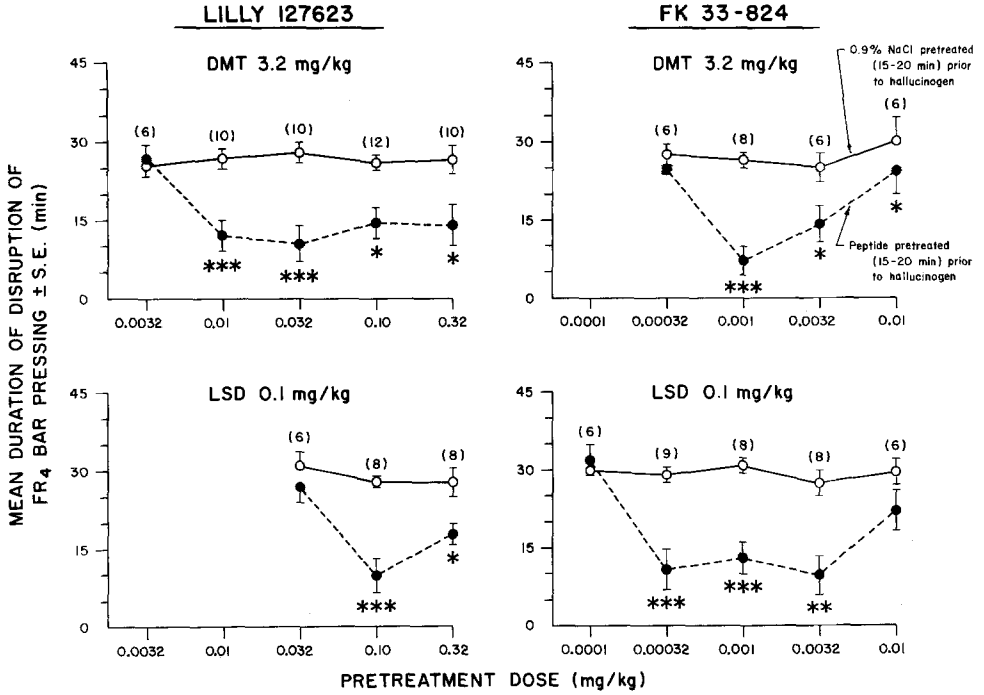

Fig. 1. Dose-dependent effects of Lilly 127623 and FK 33-824 pretreatment on the duration of DMT- and LSD-induced disruption of food-rewarded FR-4 bar pressing in the rat. In control and drug-pretreatment bar pressing sessions, designated groups ($N = 6–12$) received fixed doses of DMT (3.2 mg/kg) or LSD (0.1 mg/kg) with each rat serving as its own control. Graph points represent the mean duration (min ± SE) of FR-4 bar pressing disruption, with solid lines (○——○) representing control groups and broken lines (●– – –●) representing corresponding Lilly 127623 (0.0032–0.32 mg/kg) and FK 33-824 (0.0001–0.01 mg/kg) pretreatment groups. For control pretreatment, groups received 0.9% NaCl 15–20 min prior to DMT or LSD. In drug-pretreated groups predetermined behaviorally noneffective doses of LY 127623 and FK 33-824 were also administered at 15–20 min intervals prior to DMT or LSD. Significant antagonism to DMT-induced disruption of FR-4 bar pressing is shown with pretreatment doses of LY 127623 (0.01–0.32 mg/kg) and FK 33-824 (0.001–0.01 mg/kg) (*top*), whereas antagonism of LSD-induced behavioral effects is shown with LY 127623 (0.10–0.32 mg/kg) and FK 33-824 (0.00032–0.0032 mg/kg) pretreatment (*bottom*). Statistical significance of drug effects for control and corresponding pretreatment groups were calculated using two-tailed paired-comparison Student's *t*-tests in which the following symbols apply: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Numbers enclosed by parentheses represent the number of animals in designated groups. All injections were administered IP

induced behavioral effects is shown with doses of LY 127623 (0.10 – 0.32 mg/kg) and FK 33-824 (0.00032 – 0.0032 mg/kg). Although no differences from the DMT-induced effect were observed with pretreatment doses of 0.0032 mg/kg LY 127623 or 0.00032 mg/kg FK 33-824, and no differences from the LSD-induced effect were observed with 0.032 mg/kg LY 127623 or 0.0001 mg/kg and 0.01 mg/kg FK 33-824, it should be noted that typical gross behavioral effects characteristically observed with DMT or LSD were minimal or absent for the duration of induced disruption of FR-4 bar pressing. The data indicate LY 127623 as the more effective antagonist for DMT (3.2 mg/kg) and FK 33-824 as the more effective LSD (0.1 mg/kg) antagonist.

Discussion

The present study demonstrates complex interactions between the synthetic metenkephalin opioid peptide analogs LY 127623 and FK 33-824 and the indole hallucinogens DMT and LSD. The dramatic antagonistic dose-dependent effects of the opioid peptides reported parallel the previous findings that the opioid agonists morphine and methadone antagonized disruption of FR-4 bar pressing induced by DMT and LSD, whereas the opioid antagonists naloxone and naltrexone potentiated the effects of DMT and LSD (Ruffing and Domino 1979, 1981).

Evidence implicating endogenous ligands and opioid receptors in the pathogenesis of various psychiatric illnesses has been reported by numerous investigators (Usdin et al. 1979; Olson et al. 1981; Gacel et al. 1981). As a model for psychosis, DMT and LSD-25 were used because they induce and mimic various aspects of psychotic behavior (Stoff et al. 1978; Jenner et al. 1980; Shulgin 1981). While DMT has been identified as an endogenous substance (Smythies et al. 1979), which can be synthesized from tryptamine in various tissues of humans and animals (Saavedra et al. 1973), significant differences in the amount of DMT excreted by psychotic and normal individuals have also been demonstrated (Erdelyi et al. 1979; Murray et al. 1979). Similarly, LSD-displacing factors (LDF) have been reported in unmedicated psychotic patients (Mehl et al. 1977) while, in rat brain endogenous ligands for two distinct LSD-serotonin receptors have recently been demonstrated (Peroutka et al. 1981).

It has been reported that DMT and LSD, while sharing similar hallucinogenic properties, show differences in the extent of dopaminergic and serotonergic involvement and activity (Glennon et al. 1980; Glennon and Rosecrans 1981; Nichols 1981). Prominent among LSD pharmacological and biochemical effects is a dual ability both to mimic and to block serotonin (5-HT) in the brain (Jacobs and Trulson 1981) and also to interact with dopamine (DA) and 5-HT receptors (Rosenfeld and Makman 1981). In addition, LSD has been shown to be a potent direct-acting DA agonist, whereas DMT is without effect (Cristoph et al. 1977; Von Hungen et al. 1975). Recently, binding studies have demonstrated that ^{3}H-LSD can attach both to DA and 5-HT neurotransmitter sites, including spiroperidol-sensitive DA sites (Nielsen et al. 1980; Peroutka et al. 1981). The reported DA agonist activity is unique for LSD since other hallucinogens, like DMT, mescaline, and psilocybin have not demonstrated similar dopaminergic agonist actions (Burt et al. 1976; Jacobs and Trulson 1979, 1981; Trulson et al. 1981). The synergistic effect of an increase in the dopaminergic activity combined with a decrease in serotonergic activity may be a major factor in hallucinogenic drug potency, with the DA agonist activity modulating the magnitude of drug response.

Although DMT and LSD similarly decrease 5-HT turnover, only LSD has been shown to increase brain norepinephrine (NE) turnover and also alter the response of both 5-HT- and catecholamine-stimulated adenylate cyclase, whereas DMT alters only the catecholamine-stimulated response (Ahn and Makman 1979). Interestingly, morphine-like opioids have been shown to inhibit stimulated adenylate cyclase activity (Terenius and Wahlstrom 1978).

The relationship of opioid peptides and synthetic analogs in psychological homeostasis is becoming increasingly important in view of their behavioral effects and high specificity of endorphin actions on brain functions (Frederickson et al. 1980; Fuxe et al. 1980; Verebey 1981). The existence of multiple neurotransmitters and opioid receptors has been clearly documented, indicating important variations in the molecular mechanism of action (Morley 1980; Beddell et al. 1980; Martin 1981).

The data obtained in these investigations substantiate important interactions of indole hallucinogens with opioids in the rat and strengthen the evidence for involvement of endogenous opioids in the pathogenesis of psychoses. The dramatic dose-dependent hallucinogen antagonistic effects of LY 127623 and FK 33-824 are especially significant and parallel previous findings involving interactions of morphine and methadone with DMT and LSD. These findings suggest that opioid agonists and related opioid-like peptides antagonize the effects of DMT and LSD by a specific and selective opioid agonist action. It would be of interest to extend these findings using various behavioral paradigms to examine effects of chronic pretreatment of opioid agonists and related peptides, and possible effectiveness of opioid antagonists to reverse the antagonistic effects of opioid agonists on hallucinogen-induced behavior.

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