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Psychopharmacology 64, 31–34 (1979) Wi. 1

Psychopharmacology

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Drugs that Antagonize Limb Flick Behavior Induced by D-Lysergic Acid Diethylamide (LSD) in Cats

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Abstract. In cats observed in their home cages limb flicks (LF) are a sensitive measure of the behavioral effects of LSD. LF induced by LSD (50 µg/kg) were blocked by dextrorphan (0.6 mg/kg), dextromethorphan (0.6 mg/kg), and imipramine (5 mg/kg) at doses that did not produce ataxia or sleep. Levorphanol (0.6 mg/kg), a narcotic that is a congener of dextrorphan, did not block LF induced by LSD possibly because it produced an excitatory effect when given alone. Pentobarbital at low doses (2 and 4 mg/kg) increased the number of LF induced by LSD but at a high dose (8 mg/kg) decreased LF induced by LSD either by producing ataxia, so the cats tended to remain immobile, or by producing sleep. Chlorpromazine (CPZ) at three doses (2, 4, and 8 mg/kg) attenuated the effects of LSD on LF, but did not block LF as completely as the above three blocking drugs, and produced ataxia and sleep.

Key words: LSD antagonists – Dextrorphan – LSD – Imipramine – Dextromethorphan – Behavior – Cats – Pentobarbital – Chlorpromazine

There is evidence that narcotics (e.g., morphine) and LSD interact because LSD blocks some physiological effects of morphine. LSD (50–500 µg/kg) blocked morphine analgesia (as measured on the hot plate) in the rat (Dhawan and Gupta, 1959) and, at 25–50 µg/kg, also blocked emesis induced by morphine and apomorphine in dogs (Dhawan and Gupta, 1961). The converse is also true in that morphine blocks some physiological effects of LSD. Morphine blocked pyrexia induced by LSD in rabbits (Dhawan, 1960) and hypotension induced by LSD in dogs (Gupta et al., 1968). The latter effect was due to an action of these drugs in the brain since both LSD (0.3 mg) and morphine (10 mg) were administered intraventricularly. Since morphine blocks some of the physiological effects of LSD it is possible that morphine and related drugs, both narcotic and nonnarcotics, might block behavioral effects of LSD.

The purpose of the present study was to measure the ability of drugs from four different pharmacological classes to block the behavioral effects of LSD in cats as measured by limb flicks (LF), one of the most sensitive measures of behavioral effects of LSD in the cat (Jacobs et al., 1976, 1977; Trulson and Jacobs, 1977). We used the opiate agonist levorphanol, dextrorphan (an isomer of levorphanol), and dextromethorphan to determine if any of these drugs would alter LF produced by LSD in the cat. Dextrorphan and dextromethorphan lack narcotic activity. Three other drugs (imipramine, chlorpromazine, and pentobarbital) were included in this study as representatives of three other drug classes to compare their ability to block LF induced by LSD with the effects of dextrorphan, dextromethorphan, and levorphanol. Imipramine, a tricyclic antidepressant, attenuates the excitatory effects of LSD on the acoustic startle response in rats (Davis et al., 1977). Chlorpromazine, a phenothiazine, is frequently used to treat crises produced by psychedelic drugs (McCabe, 1977). In addition, sedating drugs (e.g., short-acting barbiturates) are also effective in treating the effects of hallucinogenic drugs. The sedative effects of phenothiazaines may explain their usefulness in the treatment of acute behavioral problems associated with hallucinogenic drugs (McCabe, 1977).

Materials and Methods

General. Ten adult, mongrel female cats (2.5–3.5 kg) were used. Observation periods in home cages lasted for 1 h. The home cage was a stainless steel structure (60 × 74 × 44 cm) with two perches, one 24 cm and the other 44 cm from the floor. Cats were allowed at least 6 weeks in these home cages to habituate before drug studies in the home cage were started.

0033-3158/79/0064/0031/$01.00

Two injections (either IM or IP) were given 15 min prior to the observation period. A putative antagonist drug was given in one injection immediately prior to an injection of LSD. During nondrug sessions saline was given in two injections (one IM and one IP) to control for the behavioral effects of injections per se. Drugs were dissolved in sterile isotonic saline. The drug dose (as the salt) and the route of administration were as follows: LSD (50 µg/kg, IP); d-2-bromo-lysergic acid diethylamide (BOL) (50 µg/kg, IP); levorphanol tartrate (Lev) (0.6 mg/kg, IP); dextrorphan (Dex) (0.6 mg/kg, IP); dextromethorphan (DM) (0.6 mg/kg, IP); imipramine hydrochloride (IMI) (5 mg/kg, IM); sodium pentobarbital (Pent) (2, 4, and 8 mg/kg, IP); and chlorpromazine (CPZ) (2, 4, and 8 mg/kg, IM). The range of injection volumes for each of the following is given in parentheses after the drug. LSD (0.5 ml/kg); BOL (0.5 ml/kg); Lev (0.5 ml/kg); Dex (0.5 ml/kg); DM (0.5 ml/kg); IMI (0.4 ml/kg); Pent (1 ml/kg); and CPZ (0.1 ml/kg).

Behavioral Assessment. The number of LF shown by each cat was counted during a 1 h observation period which began 15 min after drug administration. The observer was 'blind' with respect to the identity of the drugs administered. LF were scored when the cat lifted her paw and shook it vigorously as if she had stepped in water. In the animal model described, LF were the best measure of the behavioral effects of LSD for the following reasons: It was easy to score because its occurrence is unambigious; it was the most sensitive and reliable of the behaviors measured; it occurred at a high frequency; it was observed in all animals tested; it was the most sensitive measure of tolerance; and its occurrence after LSD administration paralleled the duration of action and tolerance that occurs in humans (Jacobs et al., 1977; Trulson and Jacobs, 1977).

Statistical Analysis. Since the incidence of LF was close to zero under control conditions, the variances were not homogeneous and the measure was not normally distributed. Thus, a nonparametric statistical test, the Wilcoxon signed rank test (Spence et al., 1976), was used. The statistical criterion for this study was $P < 0.05$.

Results

LF as a Measure of Behavioral Effects of LSD. When one dose of LSD (50 µg/kg) was administered cats reliably showed an increase in LF (Table 1). Some cats showed a high number of LF and others very few after LSD; however, all cats showed at least one LF after LSD. BOL, which has considerably less hallucinogenic potency than LSD (Isbell et al., 1959), did not produce LF at 50 µg/kg (Table 1). Limb flicks only occurred after administration of LSD and did not occur after administration of saline, Dex, DM, IMI, and Pent (Table 1).

Blockade of LSD-Induced LF by Dex, DM, and IMI. Dex, DM, and IMI all blocked the LF produced by LSD (Table 1). When these drugs were administered with LSD they produced a significant decrease ($P < 0.01$) in LF relative to LSD alone. Furthermore, there was no significant difference in LF when the three drugs (Dex, DM, and IMI) were used with LSD and when the cats had received no drug. In contrast, when Lev and LSD were used together there was no difference in the LF measure (Table 1). In general cats treated with Dex, DM, and IMI along with LSD appeared very similar to saline controls; the cats moved easily around the cage with no apparent disturbances in gait or balance.

Effect of Pentobarbital and CPZ on LSD-Induced LF. In contrast to the effects obtained with the three drugs described above, cats treated with Pent or CPZ were usually ataxic and frequently fell off their perches. When administered in conjunction with LSD the effect of Pent on LF was dose-dependent. At two lower doses (2 and 4 mg/kg) the frequency of LF after LSD increased (Table 1), while at a higher dose of Pent (8 mg/kg) cats appeared sedated; LF decreased as locomotor behavior decreased and sleeping increased. In cats treated with CPZ the nictitating membrane usually covered half the eye. After falling off the perches several times the cats lay quietly on the bottom of the cage, only occasionally staggering over to drink

Table 1. Blockade of LSD-induced limb flicks (LF) in cats by various drugs. This table compares the effects of drugs and saline given alone to their ability to block the effects of LSD on LF behavior. The number of animals used for each observation is 10 unless otherwise noted. Means are followed by ± 1 SEM unless $N = 2$, in which case both observations are given

Drug in combination with saline	Number of LF	Drug in combination with LSD (50 µg/kg)	Number of LF
LSD (first observation)	15.2 ± 3.5	Dextromethorphan	5.1 ± 2.0[b]
LSD (second observation)	16.7 ± 1.6	Dextrorphan	3.6 ± 1.7[b]
2-bromo-LSD	0.5 ± 0.2	Imipramine	2.6 ± 1.6[b]
Dextromethorphan	0	Levorphanol	12.1 ± 0.28
Dextrorphan	0	Pentobarbital (2)[a] ($N = 4$)	15.3 ± 3.6
Levorphanol	0	Pentobarbital (4)[a] ($N = 2$)	15.0; 25.0
Chlorpromazine	0	Pentobarbital (8)[a] ($N = 4$)	5.8 ± 2.5
Saline	0	Chlorpromazine (2)[a] ($N = 2$)	14; 10
		Chlorpromazine (4)[a] ($N = 4$)	3.8 ± 1.4
		Chlorpromazine (8)[a] ($N = 2$)	1.0; 0.0

[a] Indicates the dose of the drug in mg/kg; for the doses of other drugs, see text

[b] Significant ($P < 0.01$) decrease in number of LF in comparison with the number of LF produced by LSD alone

from their water bowl. After a high dose of CPZ (8 mg/kg) the cats spent much of the observation period asleep. However, at all doses used, CPZ attenuated the number of LF produced by LSD (Table 1). CPZ (2, 4, and 8 mg/kg) and Pent (2, 4, and 8 mg/kg) were not tested at all doses in all cats because they were used only to determine if they could block the LF induced by LSD without affecting locomotor behavior or producing sleep. At all doses of CPZ and Pent used the cats showed ataxia, a decrease in locomotor behavior, and sometimes sleep.

Discussion

LF as a Measure of Behavioral Effects of LSD. Although the number of LF after LSD was variable from cat to cat, in agreement with a previous report all cats showed at least one LF after LSD (Jacobs et al., 1977). LF is a behavior specific for the central effects of LSD because BOL, which has no hallucinogenic activity in humans (Isbell et al., 1959), produced no increase in LF. This is in agreement with two previous reports (Jacobs et al., 1976, 1977).

Blockade of LSD Effect on LF by Dex, DM, and IMI. Dex, DM, and IMI blocked the appearance of LF produced by LSD. Furthermore, these drugs had this blocking effect at doses that did not produce ataxia, disturbances in balance, or sleep. The mechanism by which these drugs had this blocking effect is unknown although several possibilities exist. One difficulty is that the mechanism of LSD action is not fully known. One possibility is that LSD inhibits the firing of serotonin-containing neurons in the midbrain raphe nuclei, thus releasing neurons that receive a serotonergic input (i.e., postsynaptic neurons) from a tonic inhibition (Haigler and Aghajanian, 1974). Assuming that this hypothesis is correct in accounting for the increase in LF induced by LSD there are two possible sites where antagonistic drugs could act: The presynaptic serotonergic cells or postsynaptic cells in the serotonergic system (for further details, see Haigler and Aghajanian, 1974). Presumably, any drug action that would prevent acceleration in firing of the postsynaptic cells would also block the behavioral effects of LSD. This could be achieved if a drug prevented LSD from inhibiting the presynaptic cells or if a drug depressed neuronal firing in the postsynaptic cells so that they would not increase firing after systemic administration of LSD. In the cat, single unit firing in the dorsal raphe was inhibited by LSD (10–50 µg/kg) and the inhibition was associated with an increase in LF and abortive grooming (Trulson and Jacobs, 1978). Dex and DM both inhibited firing in some neurons in both the dorsal raphe and postsynaptic areas of the serotonergic system (Haigler, 1978). Thus, Dex and DM might block the behavioral effects of LSD by blocking the acceleration of neuronal firing in the postsynaptic neurons of the serotonergic system. This possibility remains to be tested.

Tricyclic antidepressants (e.g., IMI) may block the behavioral effects of LSD in rats by enhancing the depressant effects of serotonin on postsynaptic cells, thus depressing these cells and preventing them from being accelerated (Davis et al., 1977). The three drugs (Dex, DM, and IMI) may prevent increase of neuronal firing in postsynaptic cells in the serotonergic system produced after the systemic administration of LSD, and thus share a common mechanism of action. It remains to be determined how these three drugs block the behavioral effects of LSD in cats and whether the effects of LSD on the startle response in rats are blocked by Dex and DM as they are by the tricyclic antidepressants (Davis et al., 1977).

Blockade of LF with CPZ and Pent. One precaution in evaluating the ability of a drug to block LF in cats treated with LSD is that the blocking drug should not produce a change in locomotor behavior or in the dependent variables when given alone. If it does the apparent antagonistic effects may be a consequence of these changes. Pent and CPZ produced significant ataxia, disturbances in balance, apparent drowsiness, and sleeping in the cats. Pent at low doses did not block, and apparently potentiated, the effects of LSD perhaps because this drug may produce excitement at low doses (Goodman and Gilman, 1975). At a higher dose, Pent apparently attenuated the responses by incapacitating the cats and putting them to sleep. CPZ, like Pent, also produced ataxia and disturbances in balance. However, unlike Pent, it did not potentiate LF induced by LSD. At higher doses it also produced sedation. Both of these drugs apparently blocked LF induced by LSD in cats. However, this effect should be interpreted with caution since the cats were severely impaired in their locomotor behavior at all doses, therefore some of the behavioral interactions may have been masked by the cat's inability or reluctance to move.

Acknowledgements. This study was supported by the National Institute on Drug Abuse grant number 1-R01-DA-01344-3. We thank NIDA for supplying the LSD and BOL used in this study; Hoffmann-La Roche for supplying the dextrorphan, dextromethorphan, and levorphanol; and Smith, Kline and French, Ltd. for the chlorpromazine used. We also thank Dr. E. C. Hall for his help in statistical analysis of this data and Dr. Larry E. Byrd for helpful suggestions concerning the manuscript.

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Received September 18, 1978; Final Version January 2, 1979