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Pilocarpine, a Non-hallucinogenic Cholinergic Agonist, Elicits Limb Flicking in Cats[1]

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MARINI, J. L. *Pilocarpine, a non-hallucinogenic cholinergic agonist, elicits limb flicking in cats.* PHARMAC. BIOCHEM. BEHAV. 15(6) 865–869, 1981.—The centrally- and peripherally-acting muscarinic cholinergic agonist pilocarpine (PILO, 0.125–1.0 mg/kg, IP) elicited a significantly increased frequency of occurrence of limb flicking at 0.25–1.0 mg/kg, and significantly increased the frequency of occurrence of other grooming behaviors, in 4–6 cats in the 90 min following its administration. These effects of PILO at 0.5 mg/kg were antagonized by the peripherally-acting antimuscarinic agent, *N*-methylscopolamine (MESCO, 0.5 mg/kg, IP), when MESCO was administered 15 min before PILO. The same MESCO pretreatment did not significantly antagonize the behaviors when they were elicited by LSD (0.01 mg/kg, IP) or lisuride (LIS, 0.05 mg/kg, IP). These results provide further evidence that a cat behavior model for LSD-like hallucinogens which employs limb flicking and similar grooming behaviors is not specific for hallucinogens; indicate that important "model behaviors" may be elicited by a peripheral mechanism; and show that a peripheral muscarinic cholinergic mechanism is not responsible for LSD- and LIS-elicited limb flicking. The results also suggest that the increased frequency of occurrence of the model behaviors after PILO reflects their function as grooming behaviors, elicited by PILO's intense cholinergic effects, including salivation or sialorrhea and emesis.

Pilocarpine *N*-Methylscopolamine LSD Lisuride Cats Limb flicks Hallucinogens

HALLUCINATION-producing drugs or plant extracts have been studied by pharmacologists for at least a century [5,6], but assumed their greatest theoretical and potential practical importance following the discovery of the highly potent and specific hallucinogen, *d*-lysergic acid diethylamide (LSD). Since direct physiological and neurochemical analysis of the central nervous system (CNS) effects of drugs are impossible in man for ethical reasons, and because basic clinical pharmacology of hallucinogens remains handicapped by the legal and political repercussions of previous widespread recreational use of these drugs, animal models for the study of such substances are of special importance to neuroscientists.

One such animal model has been presented by Jacobs, Trulson, and their coworkers [2–4, 14, 15], who reported that acute administration of LSD and related hallucinogens to the cat, and only of such substances, increased the frequency of occurrence of several grooming behaviors, most notably limb flicking, in the 60 min following their intraperitoneal (IP) administration. Moreover, the lowest doses effective in the cat were similar to those effective in man, and administration of a second dose within 6–72 hr produced nearly complete tolerance, analogous to the human experience. The criterion for activity of a drug in this model is that acute doses produce statistically significant, dose-dependent increases in the frequency of limb flicking, with or without increased frequencies of some other drug-responsive behaviors. Because of its excellent presentation [2–4, 14] and its reliability in appropriately-housed cats (see [17]), this model appeared ideally suited for an investigation of the neuropharmacology of drug-induced hallucinosis.

However, the model's specificity for hallucinogens has recently been seriously challenged. For example, the non-hallucinogen lisuride (LIS), a *d-iso*-lysergic acid analog of LSD, meets the model's criterion for activity at very low doses, whether animals are observed in their home cages [8,9] or in a separate chamber [17]. In relatively low doses, the clinically-used LSD derivative, methysergide, a non-hallucinogen, not only elicits limb flicking and related model behaviors, but shows LSD-like tolerance, and cross tolerance to LSD [10]. Finally, high doses of apomorphine and of quipazine elicit limb flicking in cats observed in a chamber different from their home cages [17].

Although compelling evidence against the model's specificity, these results can be questioned on various grounds.

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While not hallucinogenic even after high doses in many human studies, lisuride does produce visual hallucinations after repeated administration to some Parkinson's Disease patients [10,12]. Chronic administration of methysergide has also occasionally been reported to produce hallucinations [1], and it is claimed to have been abused as an LSD substitute [11]. The doses of apomorphine active in eliciting limb flicking may be too high to be comparable to doses used in man [17], and at present there are insufficient published data on the human psychopharmacology of quipazine for an assessment of its hallucinogenicity to be made. These considerations indicate that it is desirable to have a demonstration of the model's lack of specificity which is free from such objections.

In addition, it has been suggested that only serotonergic and/or dopaminergic mechanisms are responsible for drug-induced elicitation of the model behaviors (e.g., [15]), and all of the substances discussed above are serotonergic and/or dopaminergic agents. Hence, demonstration of activity of a drug from a different pharmacological class would be of interest.

Accordingly, I am reporting here studies on the behavior of cats given clinically relevant doses of the non-hallucinogenic muscarinic cholinergic agonist pilocarpine (PILO); the effects on PILO-elicited behaviors of pretreatment with the peripherally-acting antimuscarinic agent, *N*-methyl-scopolamine (MESCO); and the effects of MESCO pretreatment on LSD-and LIS-elicited limb flicks and other model behaviors.

METHOD

Animals and Conditions

Eight healthy adult mongrel cats (5 males, 4.0–7.5 kg; 3 females, 2.6–3.7 kg) were used; previous studies showed no sex difference in the responses of cats to LSD (B. Jacobs, personal communication) or LIS [8] with respect to the behaviors scored for this study. Six of the cats had received LSD, LIS and methysergide in previous experiments [8–10]. All cats had been housed in the standard stainless steel cages (50 cm high × 50 cm wide × 60 cm deep) for at least two months prior to the studies reported here. The cages contained dishes for food (Purina Cat Chow) and water, which were available ad lib, and a litter pan and elevated wooden perch. The animal room was maintained on a 12-hr light-dark cycle (lights on: 7 a.m. to 7 p.m.), at a temperature of 27–29°. Routine feeding and cage maintenance were performed daily by the Division of Animal Care, Yale University School of Medicine.

Drugs

All experiments employed IP injections of physiological saline (0.25 or 1.0 mg/kg) or of drugs dissolved in saline. Pilocarpine (PILO) was used as the HCl salt (Sigma), and administered at doses of 0.125–1.0 mg/kg (0.25 ml/kg); these doses correspond to 0.11–0.85 mg/kg of the free base (0.51–4.1 μmol free base/kg). N-Methylscopolamine (MESCO) was used as the Br salt (Sigma) at a dose of 0.5 mg/kg (0.25 ml/kg), corresponding to 0.40 mg/kg of the active drug (1.3 μmol/kg). *d*-Lysergic acid diethylamide (LSD), as the bitartrate (National Institute on Drug Abuse, NIDA), was used at a dose of 0.01 mg/kg (1 ml/kg), corresponding to 7.5 μg of free base/kg (23 nmol/kg). Lisuride (LIS), as the hydrogen maleate (Schering AG), was used at a dose of 0.05 mg/kg (1 ml/kg), corresponding to 38 μg of free base/kg (110 noml/kg). All solutions were prepared daily except for LSD, which was obtained from a stock solution (0.10 mg/ml in saline) stored at room temperature in the dark. Atropine sulfate (0.04 mg/ml) was available as an antidote for PILO.

Experimental Design and Scoring

Each animal was tested at the same time of day (10 a.m. to 4 p.m.), all cats were scored in their home cages, and at least six days elapsed between consecutive experiments with each cat. Behaviors after saline, LSD and MESCO plus LSD were socred with a knowledge of drug and dose. For the PILO dose-response study, the rater was blind to dose at all but 0.5 mg/kg, which was scored non-blind in order to evaluate the drug's behavioral activity. For the MESCO plus PILO experiment, cats were tested on two consecutive weeks. Each week they received two injections, separated by 15 min. The first was always MESCO, 0.5 mg/kg, and the second either PILO, 0.5 mg/kg, or saline. The rater was blind to the second drug given. In the experiments with LIS and LIS plus MESCO, cats were tested as described for PILO plus MESCO, with the rater blind to the second drug administered (LIS, 0.05 mg/kg, or saline), the first being MESCO. The LIS dose was chosen because it produced the highest occurrence of parasympathomimetic signs, including tearing, rhinorrhea and salivation [8]. The LSD dose was chosen to be high enough to elicit a significantly increased frequency of limb flicking, and low enough to be maximally vulnerable to antagonism by MESCO. Few parasympathomimetic effects are seen after low LSD doses [2–4, 8].

The behaviors reported on here were scored in 15-min epochs for 90 min post dose; when two injections were given, behaviors were scored for 90 min following the second injection. Scoring criteria were reported earlier [2–4, 8]. All experiments were scored by the author.

Statistics

The paired, 2-tailed *t*-test was used for all statistical comparisons. The measure of variance reported here is ±the standard error of the mean; $p<0.05$ is the criterion for statistical significance.

RESULTS

During the 90 min following the administration of 0.125 to 1.0 mg/kg of PILO, the animals showed a variety of parasympathomimetic effects, which were minimal or absent at the lowest, but often striking at the highest, dose. The principal effects included emesis, rhinorrhea, urination, defecation, lacrimation, pronounced salivation or marked, persistent sialorrhea, abdominal contractions, gagging, back arching, and piloerection; acute respiratory distress was observed in some cats at 1.0 mg/kg. Vomition, micturition, and defecation were seen within 1–10 min following PILO; salivation was generally first observed from ca. 5–30 min after dose. No incoordination or ataxia were observed, and the cats were awake throughout the observation period. Although I did not employ atropine antidotally in any experiment, it is my impression that doses of PILO much greater than 1 mg/kg should be employed with caution, with an antimuscarinic agent available.

The effects of PILO doses on the model behaviors are shown in Table 1. Comparisons of the frequencies of occurrence of the behaviors after single PILO doses and after saline showed that limb flicking was significantly increased

TABLE 1

RESULTS OF PILOCARPINE DOSE-RESPONSE AND PILOCARPINE-N-METHYL-SCOPOLAMINE ANTAGONISM EXPERIMENTS

(No. of cats) Drug and Dose, mg/kg*	Mean Occurrences/90 min ± S.E. Limb Flicks	Grooms	Abortive Grooms	Head + Body Shakes
		Pilocarpine		
(6) Saline	0.3 ± 0.2	4.0 ± 2.6	0	1.0 ± 0.49
(4) PILO, 0.125	6.8 ± 2.8	49 ± 16‡	0.8 ± 0.6	13 ± 5.0
(4) PILO, 0.25	11 ± 2.7‡	45 ± 19	0	22 ± 13
(6) PILO, 0.50	26 ± 5.8¶	50 ± 27	1.8 ± 0.77‡	27 ± 7.7§
(4) PILO, 1.0	26 ± 4.3¶	46 ± 39	1.3 ± 0.87	30 ± 19
		Pilocarpine Plus N-Methylscopolamine†		
(4) PILO, 0.50	29 ± 4.5¶	29 ± 5.8§	1.5 ± 1.0	24 ± 8.8
(4) PILO, 0.50 + MESCO, 0.50	3.3 ± 1.7	6.3 ± 5.4	0	6.3 ± 4.2
(4) MESCO, 0.50	2.5 ± 0.75§	1.5 ± 0.75	0	6.5 ± 6.4
(4) Saline	0.3 ± 0.3	2.5 ± 2.1	0	0.5 ± 0.3

*PILO=pilocarpine; MESCO=N-methylscopolamine. All drugs administered IP.
†MESCO administered 15 min before PILO.
Statistics: Results vs saline: ‡$p<0.05$; §$p<0.02$; ¶$p<0.01$. Underscored values: PILO vs PILO + MESCO: Limb flicks, $p<0.01$; Shakes, $p<0.05$.

at most doses, and the other behaviors after one dose. The large variance in the grooming and shaking scores (see Table 1) probably contributed to the absence of significant t-ratios at most doses. The 0.5 mg/kg PILO dose gave a significantly increased rate of limb flicking relative to the 0.125 mg/kg dose, $t=3.27$, $df=3$, $p<0.05$. The effects of PILO on most behaviors appears maximal at about 0.5 mg/kg. After saline, only one of six cats limb flicked; all cats limb flicked at least four times after PILO.

MESCO, 0.5 mg/kg, produced pronounced, very persistent mydriasis, and behaviors interpretable as secondary to dryness of the mouth: Chop-licking, pawing of the perioral area or oral cavity, and frequent jaw opening. Some animals slept during part of the 90-min scoring period following MESCO administration. In six cats that received MESCO at least once, vomition, salivation, urination and defecation were not observed. When MESCO was administered 15 min prior to PILO in four cats, no parasympathomimetic signs were observed, but all cats showed both mydriasis and brief periods of "dryness of the mouth," which began from 40–47 min post-PILO, as indicated by jaw opening and pawing of the oral cavity.

MESCO pretreatment also significantly antagonized PILO's elicitation of limb flicking and shaking, and reduced scores for grooming and abortive grooming (Table 1). The frequency of occurrence of limb flicking was significantly greater following MESCO in control experiments than following saline. To minimize the variance in limb flicking and grooming scores, the four cats used in the MESCO-PILO experiment were chosen from the six that received 0.5 mg/kg of PILO, by omitting one animal with a very high grooming score (173 grooms per 90 min), and one animal with a low limb flicking score (4 limb flicks per 90 min).

After LSD or LIS, the gross behavior of the animals was as described previously [2–4, 8, 10], and one or both drugs gave significantly increased frequencies of occurrence of all of the model behaviors relative to saline control (Table 2). Neither LIS nor LSD antagonized MESCO-induced mydriasis; MESCO blocked LIS-elicited rhinorrhea, salivation, or lacrimation in a few cats in which these signs were observed after LIS. MESCO pretreatment had no significant effect on LSD- or LIS-elicitation of model behaviors (Table 2). However, MESCO non-significantly reduced the frequency of grooming: MESCO plus LSD vs LSD, $t=2.38$, $df=5$, $p<0.07$; MESCO plus LIS vs LIS, $t=2.28$, $df=5$, $p<0.08$.

DISCUSSION

Muscarinic cholinergic agents, of which pilocarpine and muscarine are prototypical, are not known as hallucinogens in human pharmacology, and even toxic syndromes due to overdoses of such agents are not characterized by hallucinogen-like symptoms [7,13]. Not only are the muscarinic agonists as a class non-hallucinogens, but their specific pharmacological antagonists, the antimuscarinic or atropinic drugs, possess such properties, which are well known, e.g., for scopolamine. Indeed, part of the original evidence supporting the putative specificity of the cat behavior model for hallucinogens was the absence of an increased occurrence of limb flicking and other model behaviors even after heroic doses of atropine [4]. While it is of course true that muscarine is a constituent of *Amanita muscatia*, a species used for its euphoriant and hallucinatory properties, it has long been known that muscarine plays little, if any, role in such effects [16]. A current authority states that *A. muscaria's* hallucinatory effects are due to the "anticholinergic

TABLE 2

EFFECTS OF N-METHYLSCOPOLAMINE PRETREATMENT ON BEHAVIORS ELICITED BY LSD OR LISURIDE

Drug and Dose, mg/kg*	Mean Occurrences/90 min ± S.E. for Six Cats			
	Limb Flicks	Grooms	Abortive Grooms	Head + Body Shakes
Saline	2.5 ± 1.5	9.7 ± 4.1	0	3.3 ± 1.3
MESCO, 0.50	2.3 ± 1.3	2.5 ± 1.7	0.5 ± 0.4	8.7 ± 4.0
LSD, 0.01	41 ± 12‡	31 ± 11‡	1.5 ± 0.62‡	17 ± 8.0
LSD, 0.01 + MESCO, 0.50	42 ± 10¶	11 ± 4.6	1.5 ± 0.88	27 ± 8.4‡
Saline	0.3 ± 0.2	9.2 ± 5.4	0	1.5 ± 0.71
LIS, 0.05	38 ± 21	32 ± 13	4.8 ± 1.6‡	12 ± 4.8‡
(N=5)†	20 ± 8.0‡			
LIS, 0.05 + MESCO, 0.50	31 ± 18	20 ± 10	3.8 ± 2.3	12 ± 3.2§
(N=5)†	18 ± 14			

*LSD=*d*-lysergic acid diethylamide; LIS=lisuride; other abbreviations as in Table 1. MESCO administered 15 min before LSD or LIS; all drugs given IP.

†Score for N=5 animals omits the limb flick result for one cat with 129 limb flicks/90 min.

Statistics: ‡$p<0.05$; §$p<0.02$; ¶$p<0.01$. Underscored values: LSD vs LSD + MESCO: Shakes, $p<0.05$. MESCO vs LSD + MESCO: Limb flicks, $p<0.02$; Shakes, $p<0.01$.

and hallucinogenic properties of a variety of isoxazole derivatives" [13].

Pilocarpine is used in clinical pharmacology, e.g., as a diaphoretic, for which use its dose is put at 10–15 mg/person [7,13], corresponding to 0.15–0.21 mg/kg for the standard 70 kg body, doses approximately in the range at which it was active in eliciting limb flicks in this work. The toxic dose is not known, but 100 mg/person, or 1.4 mg/kg is considered "dangerous" [7]; this corresponds roughly with my observation that 1 mg/kg caused acute respiratory distress in some cats.

The results presented above show that pilocarpine, a non-hallucinogenic drug, in doses that correspond to those used clinically, elicits a dose-dependent, significantly increased frequency of occurrence of limb flicking, the key behavior in the cat behavior model for LSD-like hallucinogens. This further demonstrates that the model is not specific for hallucinogens.

Although the CNS is undoubtly involved in the expression of limb flicking, the statistically significant antagonism of PILO-elicited limb flicking by the peripherally-acting antimuscarinic drug, MESCO, strongly suggests that PILO-elicited limb flicking has a peripheral origin. This result emphasizes that elicitation of limb flicking and similar model behaviors by drugs cannot be reliably assumed to have an origin in the CNS.

The conclusions above indicate that the use of drug-elicited limb flicking and related behaviors as a specific model for studying CNS mechanisms of hallucinogens requires, in part, a demonstration that the behaviors are not blocked by a peripherally-acting antimuscarinic agent. They also show that serotonergic and/or dopaminergic properties are not necessary for the elicitation of a high frequency of limb flicking by drugs.

The absence of antagonism of LSD- and LIS-elicited limb flicking by MESCO shows that the ergolines do not elicit this behavior through a peripheral muscarinic cholinergic mechanism. However, it is still an open question whether or not other peripheral mechanisms, e.g., serotonergic, are responsible for the increased frequency of occurrence of limb flicking after LSD and LIS.

Finally, the florid parasympathomimetic signs following PILO, and the normally fastidious behavior of the cat, emphasize that the model behaviors are, after all, grooming behaviors; perhaps this is a behavioral forest that has been overlooked for the pharmacological trees. The increased frequency of grooming/model behaviors observed after PILO may well simply reflect the animals' need to cleanse themselves after bouts of profuse salivation, vomiting, and so forth. If so, the effects of LSD and other drugs on the grooming/model behaviors may reflect the animals' responses to internal, peripherally-mediated stimuli that can trigger grooming without having other sequelae as obvious to an observer as those following PILO. Therefore, it will be important to establish the relevance of grooming behaviors *per se* to CNS mechanisms that may mediate LSD's hallucinogenic effects in man.

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