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The Behavioral Effects of Hallucinogens in Rats Following 5,7-Dihydroxytryptamine Administration into the Medial Forebrain Bundle

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COMMISSARIS, R. L., D. J. MOKLER, W. H. LYNESS, K. E. MOORE AND R. H. RECH. *The behavioral effects of hallucinogens in rats following 5,7-dihydroxytryptamine administration into the medial forebrain bundle.* PHARMAC. BIOCHEM. BEHAV. **14**(6) 915–918, 1981.—The hypothesis that 5-hydroxytryptamine (5-HT) neurons and/or receptors are involved in the mechanism of action of hallucinogens is supported by the fact that intraventricular administration of the neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) selectively destroys central 5-HT neurons in the brain and potentiates the behavioral effects of lysergic acid diethylamide (LSD), 2,5-dimethoxy-4-methylamphetamine (DOM) and mescaline. The locus in the brain where this potentiation might occur is not known. In the present experiment, the medial forebrain bundle (MFB) was studied because it is the primary tract containing fibers from the cell bodies in the raphe nuclei to forebrain structures receiving 5-HT input. Male rats received 5,7-DHT (6 μg/2 μl) or vehicle injections bilaterally into the MFB; this procedure caused a significant reduction of 5-HT in the cortex, hippocampus and hypothalamus of lesioned rats, but not in the striatum. Regional dopamine and norepinephrine concentrations were not affected by this treatment. The behavioral effects of the hallucinogens were tested in a situation in which the animals pressed a bar under a fixed ratio-40 (FR-40) schedule of food reinforcement. The disruptive effects of LSD on responding were enhanced in the 5,7-DHT-treated animals, while the effects of DOM were diminished; there was no change in the response to mescaline. These data suggest that, while 5-HT neurons are involved in the behavioral effects of hallucinogens, the precise sites and/or mechanisms of action of LSD, DOM and mescaline may differ.

Hallucinogens LSD DOM Mescaline 5-HT Medial forebrain bundle 5,7-DHT

MANY of the behavioral effects of hallucinogens may be related to the ability of these agents to interact with 5-hydroxytryptamine (5-HT)-containing neuronal systems in the brain [1–3, 7–9, 11, 13, 16–18]. One behavioral paradigm which has been used extensively in the study of these interactions in rats is bar-pressing under a fixed ratio (FR) schedule of food reinforcement [2, 3, 6–9, 11]. In this situation, decreasing central 5-HT concentrations with either systemic administration of *p*-chlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor, or the intraventricular administration of the 5-HT neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) potentiates the disruptive effects of both phenethylamine (mescaline-type) and indolealkylamine (*d*-lysergic acid diethylamide [LSD]-type) hallucinogens [2, 3, 7, 8, 11]. Although these treatments are relatively specific for 5-HT neurons, they both produce large depletions of 5-HT throughout the brain and therefore provide no information regarding the site of action of these agents. Moreover, pretreatment with *p*-chloroamphetamine (PCA), a 5-HT releasing agent [15] which produces a decrease in 5-HT which is greater in magnitude than that observed following PCPA, does not alter the FR disruptive effects of LSD [11]. Therefore, it appears that the pattern as well as the magnitude of 5-HT depletion is important in determining the behavioral response of rats to hallucinogens.

The processes of 5-HT neurons extend forward from the raphe nuclei to various forebrain structures via the medial forebrain bundle (MFB), which also contains noradrenergic and dopaminergic neurons. In animals pretreated with desipramine to prevent the uptake of the neurotoxin into norepinephrine neurons, 5,7-DHT has been shown to destroy selectively those nerve terminals receiving 5-HT input from the raphe, while sparing norepinephrine and dopamine terminals in the same ragions [14]. By selectively depleting the MFB of 5-HT-containing processes, the possible site(s) of action of the hallucinogens may be restricted to those forebrain regions receiving this 5-HT input. If these areas are involved in the effects of hallucinogens, then a change in the response to hallucinogens would be expected after pretreatment with 5,7-DHT. Therefore, the purpose of the present study was to examine the disruptive effects of the hallucinogens LSD, 2,5-dimethoxy-4-methylamphetamine (DOM) and

TABLE 1

THE EFFECTS OF 5,7-DHT ADMINISTERED INTO THE MEDIAL FOREBRAIN BUNDLE ON REGIONAL BRAIN AMINE CONCENTRATIONS

	5-HT		DA		NE	
	Vehicle	5,7-DHT	Vehicle	5,7-DHT	Vehicle	5,7-DHT
Cortex	0.42 ± 0.02	0.22 ± 0.01* (52)	n.d.	n.d.	0.22 ± 0.02	0.25 ± 0.01 (111)
Hippocampus	0.41 ± 0.02	0.20 ± 0.03* (48)	n.d.	n.d.	0.37 ± 0.03	0.34 ± 0.04 (92)
Hypothalamus	1.09 ± 0.05	0.78 ± 0.04* (71)	n.d.	n.d.	2.01 ± 0.16	1.82 ± 0.27 (90)
Striatum	0.43 ± 0.04	0.35 ± 0.10 (81)	5.12 ± 0.41	6.12 ± 0.58 (120)	n.d.	n.d.

Data are expressed as μg amine/g wet tissue weight as determined fluorometrically. Each value represents the mean ± SEM from four 5,7-DHT-treated or eight vehicle-treated subjects. Numbers in parentheses represent concentration of amine in 5,7-DHT-treated subjects expressed as percentage of vehicle-injected controls.

n.d. = amine concentration not determined.

*$p<0.05$ Student's t-test.

mescaline in subjects treated with either vehicle or 5,7-DHT into the MFB.

METHOD

Subjects

The subjects were 12 drug naive, male Sprague-Dawley (Spartan Farms, Haslett, MI) rats weighing between 180–200 g at the start of the experiment. All subjects were housed separately in a room with a 12-hour light-dark cycle (lights on 0700–1900 hr).

Behavioral Apparatus

Testing was conducted between 1300 and 1500 hr in one of four standard operant chambers (LVE No. 143-20-215) equipped with food pellet dispensers; these chambers were located in sound attenuating boxes. Each chamber contained a single lever which was activated by a force of 10–15 g. All experimental events were controlled by electromechanical programming circuits and responses were recorded on electromagnetic counters and cumulative recorders. Two parameters of operant responding were monitored: (1) the number of reinforcers obtained (a reflection of the average response rate) and (2) the period of non-responding, or "pausing." To quantify the period of non-responding a pause interval counter (6–8) was incorporated into the program.

Neurochemical Lesions

The subjects were assigned randomly to one of two groups. Each subject was pretreated with desipramine (25 mg/kg, IP), to prevent the destruction of norepinephrine neurons [4], 45 minutes prior to the beginning of the stereotaxic procedure. Anesthesia was induced with Equithesin (2 ml/kg, IP). The animals were then placed in a stereotaxic apparatus and a 30 gauge stainless steel cannula was directed bilaterally into the MFB. Coordinates used were those of König and Klippel [12]: A 2.6, L±0.6, V −7.3. Animals in the 5,7-DHT-treated group received 1 μl/min for 2 minutes of a solution of 3 μg/μl 5,7-DHT in 0.9% saline containing 1 mg/ml ascorbate, bilaterally. Control animals received vehicle injections.

Behavioral Procedure

Following recovery from surgery subjects were deprived of food and maintained at 70–80% of their free-feeding weight. Subjects were trained to bar press for food reinforcement (45 mg Noyes pellets). Animals were first trained to respond under a continuous reinforcement (CRF) schedule by auto-shaping. After rats were responding regularly on the CRF schedule, the FR schedule was introduced and gradually increased to FR-40. Daily sessions were 40 minutes in duration. Animals were run in the same cage at the same time of day seven days a week.

After the subjects had attained stable rates of responding, behavioral testing was begun and the effects of various doses of LSD (0.0125–0.2 mg/kg), DOM (0.125–2.0 mg/kg) and mescaline (6–14 mg/kg) were determined; the order of drugs and doses administered was randomized completely for each rat. Drugs were administered intraperitoneally immediately before the start of the session. All drug test days were preceded by at least three non-drug days to avoid the possibility of tolerance development.

Biochemical Assessment of the Lesion Effects

Five days after completion of the behavioral testing the animals were sacrificed by decapitation, their brains removed, and the hypothalamus, hippocampus, striatum, and cortex were dissected under gross inspection and weighed. Fluorometric procedures were utilized to analyze 5-HT in all four regions as described by Curzon and Green [10]; concentrations of norepinephrine and dopamine were fluorometrically analyzed as described by Chang [5].

Statistical Analyses

Control FR-40 response parameters and regional brain amine concentrations in 5,7-DHT-treated subjects were compared to vehicle-treated subjects using Student's t-test.

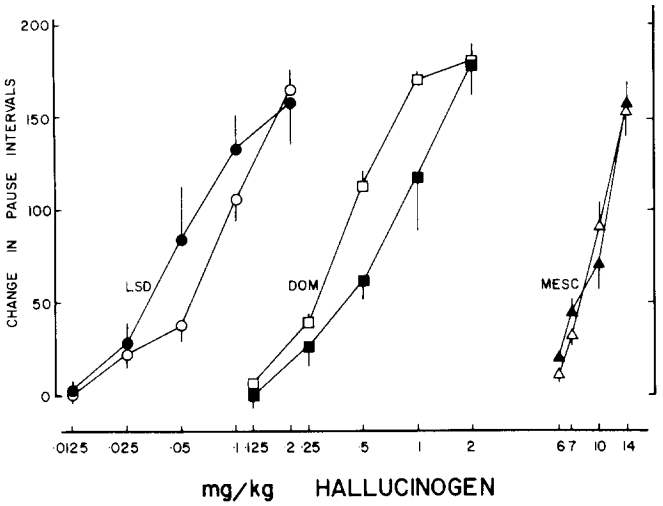

FIG. 1. Effects of LSD, DOM and mescaline on FR-40 operant responding in subjects treated with vehicle or 5,7-DHT in the medial forebrain bundle. The change in pause intervals produced by various doses of LSD (circles), DOM (squares) and mescaline (triangles) is plotted for control (open symbols) or 5,7-DHT-treated (filled symbols) subjects. Change in pause intervals was determined by comparing the values on test days to the average of the three days prior to the test day (baseline). Each symbol and vertical bar represents the mean±SEM for four (5,7-DHT-treated) or eight (vehicle-treated) subjects.

Drug effects were assessed by comparing the data from test days to the average of the three days prior to the test day (baseline). Student's t-test for paired data was used to evaluate the effects of individual doses of the drugs. Dose-response relationships for the drugs were examined by analysis of variance in a block (blocked by subjects) design. In all statistical evaluations $p<0.05$ was used as the criterion of statistical significance.

Drugs

LSD tartrate, DOM hydrochloride and mescaline hydrochloride were obtained from the National Institute on Drug Abuse. Desipramine hydrochloride was purchased from Merrell Labs (Cincinnati, OH). The 5,7-DHT creatinine sulfate was purchased from Sigma Chemical Co. (St. Louis, MO). Doses of all agents refer to the salts.

RESULTS

The effects of 5,7-DHT treatment on regional brain amine concentrations are shown in Table 1. 5,7-DHT injection into the MFB significantly decreased the concentrations of 5-HT in the cortex, hippocampus and hypothalamus. Concentrations of 5-HT in the cortex and hippocampus were reduced to nearly 50 percent of the vehicle-injected controls, while the concentration of 5-HT in the hypothalamus was reduced to 71 percent of control. Striatal 5-HT concentrations were not significantly altered by 5,7-DHT administration into the MFB. Dopamine and norepinephrine concentrations in all areas examined were not altered by this treatment.

As observed in previous studies, control FR-40 responding is characterized by a rapid constant rate of responding with brief pauses (usually occurring following the delivery of the food pellet) throughout the session, a pattern typical for this schedule [6–8]. In the present study vehicle-treated subjects received 95±5 reinforcers and produced 36±6 pause intervals in control FR-40 sessions. Administration of 5,7-DHT into the MFB did not change control FR-40 operant responding as measured by either reinforcers obtained (96±18) or pause intervals produced (48±15). Administration of the hallucinogens resulted in a disruption of FR-40 responding characterized by periods of non-responding or "pausing". Quantitation of this pausing can be seen in Fig. 1, as all three hallucinogens produced a dose-dependent increase in pause intervals. The pause-producing effects of LSD were potentiated by administration of 5,7-DHT into the MFB, $F(1,33)=8.09$, $p<0.05$, while the effects of DOM were attenuated in these animals, $F(1,33)=25.17$, $p<0.05$. The disruptive effects of mescaline were not altered by 5,7-DHT treatment into the MFB.

DISCUSSION

5,7-DHT administration into the MFB produced different neurochemical effects when compared to intraventricular injection. Injection of 5,7-DHT into the MFB produced moderate decreases in the concentration of 5-HT in the cortex and hippocampus, with only a slight decrease in the hypothalamus. There was no significant change in striatal 5-HT concentration in these animals. In contrast, intraventricular administration of 5,7-DHT has been shown to produce large (80–90 percent) decreases in 5-HT concentrations in all of these areas [8]. These differences in the pattern of 5-HT depletion produced by either intraventricular or MFB 5,7-DHT administration may be presumed to result in differences in the response to various drugs. Indeed, the response to various doses of the hallucinogens after 5,7-DHT treatment also depends on the route of administration of the neurotoxin and the quantity administered. Intraventricular injection of the neurotoxin potentiated the disruptive effects of LSD, DOM and mescaline to a similar extent [2,8]. In the present study, injection of 5,7-DHT into the MFB potentiated the effects of LSD, while the effects of DOM were attenuated and the effects of mescaline were unchanged.

Both neurotoxin treatments suggest a role of 5-HT neuronal systems in the behavioral effects of hallucinogens. Previous studies have indicated that the pattern of 5-HT depletion is critical for alterations in the FR disruptive and discriminative stimulus effects of LSD [11,16]. The present study indicates that, in subjects with a particular pattern of depletion, the effects of the various hallucinogens are differentially affected. These data are in agreement with a recent report indicating differences in antagonism of the FR-40 disruptive effects of hallucinogens by pretreatment with the putative 5-HT antagonist metergoline [9]. Thus, although interactions with 5-HT systems are strongly implicated in the behavioral effects of the hallucinogens, the above results suggest that the site(s) or mechanism(s) of action of these agents may differ.

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