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SOME BEHAVIORAL EFFECTS OF HALLUCINOGENS ARE MEDIATED BY A POSTSYNAPTIC SEROTONERGIC ACTION: EVIDENCE FROM SINGLE UNIT STUDIES IN FREELY MOVING CATS

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Although central serotonergic systems appear to be linked importantly to the mechanism of action of a variety of hallucinogenic drugs, the nature of this interaction has remained unclear. In the present study, the question of whether the critical link is presynaptic or postsynaptic was examined in cats. Behaviorally inactive doses (1.0 mg/kg) of the serotonin receptor antagonists mianserin, ketanserin or metergoline effectively blocked behavior, as measured by the cat limb flick response, elicited by either LSD (50 μg/kg) or DOM (250 μg/kg) but not that resulting either from lisuride (50 μg/kg) or a high dose of apomorphine (4 mg/kg). Pretreatment with 1.0 mg/kg of mianserin, which completely attenuated LSD's behavioral effect, failed to alter LSD-induced depression of mesencephalic serotonergic neuron discharge. These results demonstrate that at least some of the behavioral effects of LSD can be blocked by pharmacological antagonism of postsynaptic serotonin receptors which leaves LSD's presynaptic effect unaffected. Thus, the behavioral, and possibly psychoactive, effects of hallucinogens appear to be attributable to an action at $5HT_2$ receptors, presumably located postsynaptically.

Serotonin antagonists Raphe unit activity Cat behavior Serotonin receptors Hallucinogens Serotonergic neurons

1. Introduction

Understanding the mechanism of action of hallucinogenic drugs has remained elusive. Similar phenomenological effects in humans produced by LSD, psilocin, 2,5-dimethoxy-4-methylamphetamine (DOM), mescaline and N,N-dimethyltryptamine (DMT), as well as the fact that cross-tolerance can be demonstrated between many of these drugs, suggests that they constitute a distinct drug class. In turn, this suggests that their action may be mediated by a common mechanism.

Theories regarding the mechanism of action of hallucinogens have gone through several phases, often focusing primarily on the role of brain serotonin. In the initial phase in the mid-1950s, based on pharmacological analyses, LSD was hypothesized to exert its psychological actions by blocking serotonin's synaptic effects (Gaddum and Hameed, 1954; Wooley and Shaw, 1954). In the early 1960s, based on brain neurochemical studies, the experimental emphasis shifted to an effect upon serotonin in the central nervous system, but without regard to its precise site of action, i.e. presynaptic or postsynaptic (Freedman, 1961). Based on electrophysiological experiments, theories developed in the 1970s proposed that the effects of LSD, and related hallucinogens, were mediated specifically by their inhibitory action directly upon the cell bodies of serotonergic neu-

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rons, i.e. 'the presynaptic effect' (for review see Jacobs and Trulson, 1981).

Several pieces of evidence, however, have led us to question the validity of this presynaptic hypothesis. In experiments on cats we found substantial dissociations between the effects of hallucinogenic drugs on the activity of serotonergic neurons and their effects on behavior (Trulson et al., 1981). Specifically, the behavioral effects significantly outlasted the depression of serotonergic unit activity. Furthermore, when LSD was given to a cat on two successive days, very little behavioral effect occurred on the second day (tolerance), but the magnitude of the suppression of serotonergic unit activity was the same as that on the initial day of drug treatment.

More recently, emphasis has switched to the action of hallucinogenic drugs directly on the target neurons of serotonergic projections, i.e. 'the postsynaptic effect'. Several indirect lines of evidence support the view that an important aspect of the action of hallucinogenic drugs is mediated by an effect on postsynaptic serotonergic receptors. First, binding of [^{3}H]LSD, whether examined autoradiographically or using receptor specific binding assays, is unchanged by destruction of serotonergic neurons (Bennett and Aghajanian, 1974; Bennett and Snyder, 1976). Since much of this binding can be displaced by serotonin, it indicates that it is associated with serotonin receptors, but apparently not those localized on any of the various aspects of the presynaptic neuron. Second, administration of a number of different hallucinogens produces behavioral effects attributable to a direct serotonergic agonist action at postsynaptic receptor sites (Trulson et al., 1976; Sloviter et al., 1980). Furthermore, repeated administration of LSD diminishes its potency in eliciting these behavioral effects (tolerance) (Trulson et al., 1976), but is unaccompanied by a change in sensitivity of serotonergic neuron discharge to LSD (Trulson et al., 1977; 1981). However, repeated LSD administration does result in a decrease in the number of available postsynaptic serotonergic receptor binding sites (Trulson and Jacobs, 1979). Third, studies in a variety of different species, including humans, demonstrate that prior depletion of serotonin enhances the behavioral effects of hallucinogenic drugs (e.g. Appel et al., 1977; Resnick et al., 1965). In many of these cases there is direct evidence for the presence of serotonergic postsynaptic supersensitivity at the time the hallucinogens were administered. Fourth, in a variety of different paradigms, including drug discrimination, operant responding, and spontaneous behavior, the behavioral effects in rat of a number of hallucinogens can be effectively blocked by several different serotonin antagonist drugs (see Jacobs, 1983 for a review of these experiments). Finally, administration of monoamine oxidase inhibitors (MAOI) for 1-2 weeks can totally block the behavioral effects of LSD in rats, cats and humans (Lucki and Frazer, 1982; Jacobs et al., 1982b; Grof and Dytrych, 1965; Resnick et al., 1964). This blocking action may persist for as long as 2 weeks following withdrawal from the MAOI. Neurochemical evidence indicates that these decreased behavioral responses to hallucinogenic drugs may be mediated by a decrease in the availability of postsynaptic binding sites for serotonin (Savage et al., 1980; Peroutka and Snyder, 1980).

The present study continues this line of investigation. We examined the ability of several serotonin receptor antagonists to block the behavioral effects of a low dose of LSD in the cat, and whether these antagonists also blocked the depressant action of LSD on brain serotonergic unit activity. Preliminary accounts of these data have been published previously (Jacobs et al., 1982a,b).

2. Materials and methods

2.1. Animals

Adult, female cats weighing 2.5-3.5 kg were used in all experiments. The cats were given food and water ad libitum and housed individually in stainless steel cages which also served as the observation chamber for behavioral experiments. A 12:12 h light-dark cycle was maintained in the room where the cats were housed. All data collection was performed during the light portion of the cycle. The cats were allowed at least six weeks to adapt to the laboratory environment before beginning any behavioral experiments.

2.2. Behavioral experiments

The effects of various serotonin receptor antagonist drug pretreatments on spontaneous behavior (that elicited by an injection of 0.5 mg/kg saline) and on LSD-elicited behavior (50 μg/kg) were examined. The experiments were counterbalanced so that each animal received all doses of a particular pretreatment in a random order. The following serotonin receptor antagonists were utilized as pretreatments: mianserin HCl (0.0, 0.1, 0.25, 1.0 mg/kg, n = 6); metergoline (0.0, 0.25, 1.0 mg/kg, n = 6); and ketanserin HCl (0.0, 0.1, 1.0 mg/kg, n = 4). All drug doses are expressed as the salt, where applicable, and a dose of 0.0 signifies a 0.5 ml/kg injection of saline vehicle. Dilute HCl was added to the saline in order to place metergoline into solution. All injections were made by the intraperitoneal route. Except for metergoline, the cats were pretreated 30 min prior to receiving an injection of LSD or saline. Since metergoline has a long latency to the onset of its action (Beretta et al., 1965; Ferrini and Glasser, 1965), it was administered 2.5 h prior to LSD or vehicle injection. In a separate series of experiments, the effects of mianserin pretreatment (0.5 mg/kg) on behavior elicited by a single injection of apomorphine HCl (4 mg/kg, n = 4), lisuride hydrogen maleate (50 μg/kg, n = 5), or DOM HCl (250 μg/kg, n = 4) given 30 min later were examined. Because of the rapid and pronounced tolerance to LSD administration (Trulson et al., 1981), at least seven days intervened between successive observational sessions.

Behavioral observations, by raters blind to the pretreatment condition, were made during the hour immediately following treatment with LSD, vehicle, apomorphine, lisuride or DOM. During this period, behavior was tallied in 15 min blocks using a standard scoring sheet. The behaviors scored included limb flicks, abortive grooms, grooms, shakes (head and/or body), investigatory or play behavior and yawns. Most of these behavioral descriptions are self-explanatory and a detailed description of each has been previously published (Jacobs et al., 1977b). In addition, for some experiments, locomotion was scored using a procedure that we also have described previously (Jacobs et al., 1981). This quantified measure was used in combination with a rater's judgment of an animal's behavioral condition to evaluate any cataleptic, neuroleptic or sedative properties of serotonin receptor antagonists.

2.3. Electrophysiological experiments

Six cats were prepared for chronic unit recording of serotonergic neurons using a procedure that has been described in detail previously (Trulson et al., 1981). Under pentobarbital anesthesia (35 mg/kg i.p.), a microdrive, consisting of two guide cannulae, oriented in an anterior-posterior plane was stereotaxically positioned at an angle of 45° behind the vertical approaching the dorsal raphe nucleus (DRN). After the microdrive was fixed to the skull with dental acrylic, a microwire bundle, consisting of three 32 μm and three 64 μm diameter insulated nichrome wires, was lowered through each guide cannula to a point approximately 1 mm from the DRN. The anterior bundle coordinates at this point were P 1.5, L 0.0, H + 1.0 (Snider and Niemer, 1961). Gross electrodes for recording the electroencephalogram (EEG), electrooculogram (EOG), and dorsal neck electromyogram (EMG) also were implanted at this time. Four weeks were allowed for recovery from the surgical procedure before electrophysiological experiments were begun.

Electrical potentials were led from the cat by means of a counterweighted low noise cable system and 25 channel slip ring assembly. Microelectrode recordings were amplified (Grass 7P511 ac preamp), filtered (band pass 0.3-10 KHz), and monitored on an oscilloscope. Single unit activity was separated from background noise by means of a variable threshold gate-Schmitt trigger. Triggered output pulses were used to obtain an on-line record of the unit discharge through a speaker, an electronic counter, and on a polygraph. The EEG, EOG and EMG were recorded simultaneously on the polygraph record.

Serotonergic units were initially identified on-line by their characteristic slow and regular discharge. After recording 15-30 min of baseline unit activity cats received an intraperitioneal injection of either mianserin (1.0 mg/kg), or saline (0.5

ml/kg). Following 30 min of subsequent unit recording, LSD (50 μg/kg i.p.) was administered and unit recording then was continued for an additional 60 min. Verification that all recording sites were within the DRN was obtained utilizing a histological methodology that has been described previously (Trulson et al., 1981).

2.4. Data analysis

The behavioral effects of LSD were compared to saline control with paired two-tailed t-tests. The behavioral effects of apomorphine and DOM were not paired to saline controls, therefore, they were compared to an unmatched saline control group with unpaired two-tailed t-tests. The effects of pretreatment with serotonin receptor antagonists on LSD elicited behavior was compared across dose utilizing a one way analysis of variance for repeated measures. If indicated, individual comparisons between doses were performed using the Newman-Keuls test. The effects of pretreatment with a single dose of mianserin (0.5 mg/kg) on apomorphine and DOM elicited behaviors were compared to saline pretreatment with paired two-tailed t-tests. The accepted level of significance was $P < 0.05$ in all cases.

For electrophysiological experiments, a baseline firing rate was calculated for each unit by determining the mean discharge rate of six consecutive 10 s time samples during pre-drug quiet waking. Post-drug firing rates were calculated for each unit in an identical manner by using time samples at 5, 10, 15 and 30 min after mianserin pretreatment and every 15 min after LSD administration. The magnitude of drug-induced changes in the activity of individual units then was assessed by comparing unit discharge rate during the post-drug period of maximal firing rate change with the same cell's pre-drug baseline firing rate. For individual cells, a change in firing rate that exceeded 2 standard deviations from the baseline rate was considered significant ($P < 0.05$). Changes in individual unit firing rates also were expressed as percentages of baseline firing rates and a group mean percent of baseline discharge rate was calculated for saline, mianserin, saline plus LSD and mianserin plus LSD treatments. A one way analysis of variance was used to assess overall differences between these treatment groups. Comparisons between individual groups then were made at a significance level of 0.05 using the Newman-Keuls test.

3. Results

3.1. Effect of LSD on cat behavior

Consistent with previous reports from several laboratories (Jacobs et al., 1977b; White et al., 1981; Marini et al., 1981), LSD increased the frequency of occurrence of a number of behaviors in the cat. As shown in table 1, when compared with a saline injection, LSD (50 μg/kg) significantly increased the mean hourly occurrence of limb flicks, head and whole body shakes and yawns. The rate of occurrence of the other behaviors which were scored were not significantly affected by LSD administration. In agreement with previous work (Jacobs et al., 1977b), we found the limb flick to be the most reliable, robust and sensitive measure of LSD's behavioral effect in the cat. Therefore, we have focused on this behavioral measure in examining the ability of serotonin receptor antagonists to alter LSD-elicited behavior.

3.2. Effects of mianserin on spontaneous and LSD induced behavior

When administered alone, low doses of mianserin (0.1, 0.25 or 1.0 mg/kg), a serotonin receptor antagonist with a high selectivity ratio for $5HT_2$ over $5HT_1$ receptors (Peroutka and Snyder, 1981), had no significant effect on any scored behavior. However, pretreatment with these same doses of mianserin markedly attenuated the behavioral effects of LSD. Fig. 1 illustrates the effects of increasing doses of mianserin on the number of limb flicks and locomotion counts during the 1 h period immediately following either saline or LSD injection. It is clear from this graph that saline injection produced no limb flicking responses and this result was not altered by mianserin pretreatment. On the other hand, mianserin pretreatment resulted in a significant dose-dependent reduction

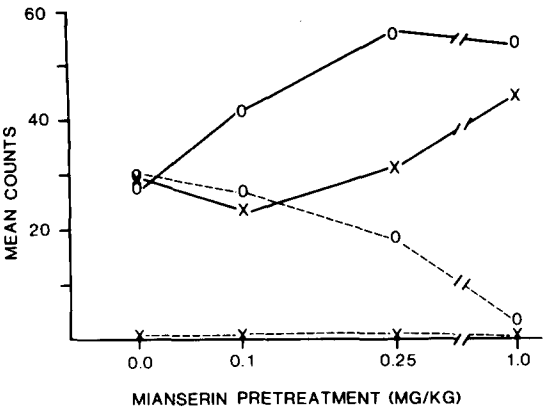

Fig. 1. Effect of mianserin pretreatment on limb flick responses (------) and locomotion (———) occurring during a 60 min period immediately following either saline (0.5 ml/kg, ×) or LSD (50 μg/kg, ○) injection. Mianserin was administered 30 min prior to saline or LSD and all injections were given i.p. Note that 0.0 mg/kg of mianserin represents pretreatment with saline vehicle alone, therefore, the corresponding behavioral scores after subsequent saline or LSD injections are baseline values. Mianserin pretreatment produced a dose dependent reduction in LSD induced limb flick behavior that was not the result of general behavioral depression since, it can be seen that during the same period, locomotor activity was somewhat elevated.

of the high limb flick rate produced by LSD (ANOVA: $F = 6.41$, d.f. = 3,15, $P < 0.01$). Mianserin doses of 0.1, 0.25 or 1.0 mg/kg reduced the LSD-elicited limb flick response by 8.4, 37.7 or 89.2%, respectively, when compared to the control LSD-induced response following pretreatment with saline vehicle (0.0 mg/kg mianserin in fig. 1). Critically important for this study is the fact that this decrease in the rate of limb flicking was not due to a general depressant action of mianserin on behavior. This is indicated by the fact that the locomotor scores, after both saline and LSD injections, were not decreased by mianserin pretreatment. On the contrary, in both cases they showed a tendency toward being elevated with increasing doses of mianserin. Furthermore, the cats displayed no observable signs of sedation, ataxia or catalepsy following mianserin pretreatment with doses up to 1.0 mg/kg. These data, therefore, preclude attributing the blocking effects of mianserin on LSD-induced behavior, that are described above, to non-specific actions or general debilitation. However, doses of mianserin greater than 1.0 mg/kg produced clear signs of sedation which were accompanied by pronounced decreases in locomotion scores. For this reason, the doses of mianserin employed in the present study were limited to 1.0 mg/kg or less.

3.3. Effects of mianserin on DOM, apomorphine and lisuride induced behavior

The ability of mianserin to block hallucinogen-induced behavior also demonstrated some specificity. Table 1 shows that a low dose of DOM (250 μg/kg), a non-indole nucleus hallucinogen, produced significant increases in the rate of limb flicking, abortive grooming and yawning. A relatively high dose of apomorphine (4.0 mg/kg), a

TABLE 1

Effects of drug treatments on spontaneous behavior in cats. All drugs were injected by the intraperitoneal route and behavior was scored during the ensuing hour by raters blind to the treatment.

Drug	Behavior (mean hourly rate ± S.E.M.)						
	Limb flicks	Abortive grooms	Grooms	Shakes	Play	Yawns	Locomotion
Saline (10)	0.4 ± 0.2	0.0 ± 0.0	10.6 ± 3.6	6.9 ± 3.4	2.3 ± 2.3	0.3 ± 0.2	21.3 ± 13.2
LSD, 0.05 * (10)	21.3 ± 4.4 [a]	0.7 ± 0.5	12.1 ± 2.5	18.0 ± 5.4 [a]	1.7 ± 0.8	2.6 ± 0.8 [a]	27.3 ± 6.3
DOM, 0.25 * (4)	51.5 ± 8.9 [b]	3.0 ± 0.8 [b]	21.3 ± 4.2	26.0 ± 14.2 [b]	1.0 ± 0.6	6.3 ± 0.5	–
APO, 4.0 * (4)	20.8 ± 1.9 [b]	3.5 ± 2.1 [b]	40.8 ± 26.1 [b]	72.3 ± 22.4 [b]	0.0 ± 0.0	0.0 ± 0.0	–
Lisuride 0.05 * (5)	26.8 ± 7.4 [b]	1.0 ± 0.4 [b]	55.4 ± 33.2 [b]	13.4 ± 6.2	0.0 ± 0.0	1.0 ± 0.8	–

* Dose, mg/kg. [a] Denotes significantly different from saline, $P < 0.05$, paired t-test. [b] Denotes significantly different from saline, $P < 0.05$, unpaired t-test. APO is the abbreviation for apomorphine; n in parentheses.

dopaminergic agonist, also resulted in significant increases in the rate of limb flicks, abortive grooms and shakes. And, an intermediate dose of lisuride (50 μg/kg) (White et al., 1981), a non-hallucinogenic congener of LSD, produced significant increases in the rate of limb flicking, abortive grooming and grooming. These results with DOM, apomorphine and lisuride are in accord with those previously reported (Jacobs et al., 1977a; Marini et al., 1981; White et al., 1981). Pretreatment with mianserin significantly reduced the number of limb flicks which occurred during the 1 h immediately after a 250 μg/kg injection of DOM. After pretreatment with 1.0 mg/kg of mianserin, DOM injection 30 min later resulted in 9.3 ± 4.8 limb flicks/h (mean ± S.E.M.) which was significantly less than the rate of 51.5 ± 8.9 flicks/h elicited by DOM injection 30 min after saline pretreatment ($P < 0.01$, paired t-test, $n = 4$). However, mianserin pretreatment, in a dose (0.5 mg/kg) that significantly reduced the number of limb flicks following LSD administration (50 μg/kg) by 47% ($n = 4$), did not influence the number of limb flicks elicited by either apomorphine or lisuride. When mianserin was administered 30 min prior to either apomorphine (4 mg/kg) or lisuride (50 μg/kg), it had no significant effect on the number of limb flicks elicited by either compound when compared to saline pretreatment (apomorphine 20.8 ± 1.9 versus 25.3 ± 7.9 flicks/h, $n = 4$; lisuride 26.0 ± 6.8 versus 26.8 ± 7.4 flicks/h, $n = 5$). Thus, the serotonin receptor antagonist mianserin blocked behavior elicited by two structurally different hallucinogens, LSD and DOM, but did not alter the same behavior produced either by a high dose of the dopaminergic agonist apomorphine, or the non-hallucinogenic ergot derivative drug lisuride.

TABLE 2

Effects of metergoline and ketanserin on spontaneous and LSD-induced behavior. Cats were pretreated with varying doses of metergoline or ketanserin 2.5 or 0.5 h, respectively, prior to either saline (0.5 ml/kg) or LSD (50 μg/kg) injection. All injections were by the intraperitoneal route. Behavior was scored during the hour immediately subsequent to saline or LSD administration by raters blind to the treatment condition. Behavior following saline injection was considered to be spontaneous behavior. Note that pretreatment with a drug dosage of 0.00 mg/kg signifies pretreatment with saline vehicle. Drug effects were compared across dose (including 0.00 mg/kg) with an analysis of variance.

Drug	Dose (mg/kg)	Spontaneous behavior (mean hourly rate ± S.E.M.)						
		Limb flicks	Abortive grooms	Grooms	Shakes	Play	Yawns	Locomotion
MET	0.00 (4)	1.3 ± 0.8	0.0 ± 0.0	7.3 ± 3.0	4.5 ± 1.0	0.0 ± 0.0	0.8 ± 0.5	1.8 ± 1.0
	0.25 (4)	0.3 ± 0.3	0.0 ± 0.0	5.8 ± 3.5	4.3 ± 2.0	0.0 ± 0.0	0.5 ± 0.3	3.8 ± 1.9
	1.00 (4)	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.2	7.3 ± 5.1	0.0 ± 0.0	0.0 ± 0.0	–
KET	0.00 (4)	0.5 ± 0.5	0.0 ± 0.0	6.8 ± 3.5	3.0 ± 1.1	0.0 ± 0.0	0.0 ± 0.0	9.3 ± 3.3
	0.10 (4)	0.3 ± 0.3	0.0 ± 0.0	7.0 ± 4.1	2.0 ± 0.7	0.3 ± 0.3	0.5 ± 0.5	7.5 ± 3.8
	1.00 (4)	1.0 ± 0.6	1.5 ± 1.5	6.0 ± 2.3	3.3 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	22.0 ± 2.3 [a]

Drug	Dose (mg/kg)	LSD-induced behavior (mean hourly rate ± S.E.M.)	
		Limb flicks	Locomotion
MET	0.00 (6)	29.7 ± 4.7	27.8 ± 8.3
	0.25 (6)	12.1 ± 3.4 [b]	49.0 ± 10.9
	1.00 (6)	4.2 ± 2.4 [b]	12.2 ± 5.4 [c]
KET	0.00 (4)	8.8 ± 1.8	26.5 ± 11.1
	0.10 (4)	3.5 ± 1.3 [b]	20.5 ± 9.9
	1.00 (4)	0.5 ± 0.3 [b]	10.5 ± 5.0

[a] Significantly different from both saline (0.00) and 0.10 dose, $P < 0.05$ (Newman-Keuls). [b] Significantly different from saline (0.00), $P < 0.05$ (Newman-Keuls). [c] Significantly different from 0.25 dose, $P < 0.05$ (Newman-Keuls). Abbreviations: MET (metergoline); KET (ketanserin); n in parentheses.

3.4. Effects of metergoline and ketanserin on spontaneous and LSD induced behavior

In order to test the generality of these results, the behavioral effects of two additional serotonin receptor antagonists were examined. Like mianserin, both metergoline and ketanserin had virtually no effect on spontaneous behavior when administered in low doses. Table 2 shows that pretreatment with 0.25 or 1.0 mg/kg of metergoline 2.5-3.5 h prior to saline injection had no significant effect on any of the scored behaviors when compared to a pretreatment-treatment regime consisting of successive saline injections. The delayed period of behavioral observation was employed because of metergoline's long latency of action. Pretreatment with 0.1 or 1.0 mg/kg of ketanserin 30 min prior to saline injection also failed to produce significant alterations of behavior scored during the hour immediately after saline injection, except for a significant increase in locomotion (table 2).

Although their effects on spontaneous behavior were minimal, metergoline and ketanserin both significantly attenuated LSD-induced behavior. It is evident from table 2 that pretreatment with either metergoline or ketanserin resulted in a significant dose-dependent reduction in the number of limb flicks elicited by LSD. This was not the result of a general depression of behavior because locomotion scores, although somewhat lower, were not significantly reduced in a dose dependent fashion following pretreatment with either drug. Although the group of cats tested with ketanserin pretreatment did not manifest a high frequency of limb flicking in response to LSD, the changes observed were highly consistent across animals (low S.E.M.s across all doses of ketanserin).

3.5. Effect of mianserin on LSD-induced depression of serotonergic neuron discharge

The next stage of this study was an attempt to determine where these serotonin antagonist drugs exerted their action in blocking the behavioral effects of LSD. Therefore, we examined whether or not the presynaptic action of LSD, as measured by LSD-induced depression of DRN serotonergic neuron discharge, was altered by pretreatment with mianserin, one of the serotonin receptor antagonists that blocked the behavioral effects of LSD. Although mianserin pretreatment produced an effective blockade of LSD-induced behavior, it was without effect on the single unit responses of serotonergic neurons to systemic LSD administration. The representative experiment shown in fig. 2 depicts the response of the same DRN serotonergic neuron to systemic LSD injection (50 μg/kg) 30 min after either saline (0.5 ml/kg) or mianserin (1.0 mg/kg) pretreatment. The unit response to LSD injection was first recorded after mianserin pretreatment and then 24 h later after saline pretreatment. The LSD injections were spaced only one day apart in order to observe the effects of LSD on the same neuron's discharge after both mianserin and saline pretreatment. Previous studies from our laboratory (Trulson et al., 1977; 1981) have shown that tolerance to the inhibitory action of LSD on serotonergic neuron discharge does not occur and therefore was not considered to be a confounding factor in the present study. This conclusion is also supported by results described below in which short interval injections were not employed. It is evident from fig. 2 that neither mianserin nor saline alone produced significant alterations in unit discharge by 30 min after their injection. Following LSD administration, the magnitude of the decrease in firing rate after mianserin pretreatment was nearly identical with the same cell's response following saline pretreatment (85.6 versus 85.0%, respectively). We found the effect of LSD on serotonergic neuron discharge rates to be identical after mianserin or saline pretreatment for a subsample of 3 cells in which both pretreatment conditions were examined on the same neuron (80.8 ± 3.9 versus 82.3 ± 2.6%, respectively). Furthermore, these results were independent from the order in which the pretreatments were given since the same results were obtained regardless of whether the saline plus LSD experiment was run before or after the mianserin plus LSD trial.

Group data demonstrating the effects on serotonergic unit discharge of administration of saline, mianserin or LSD after either saline or mianserin pretreatment are listed in table 3. Analysis of variance revealed a significant difference

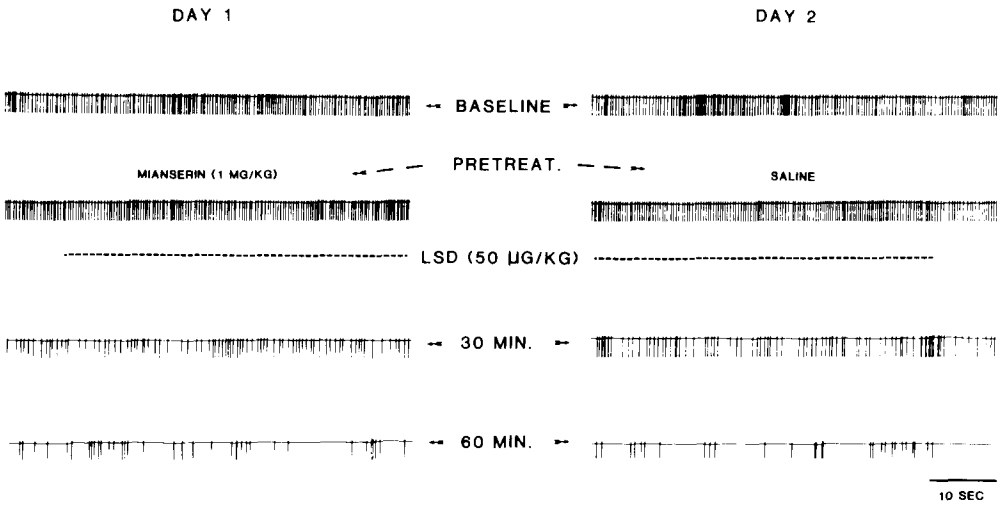

Fig. 2. Lack of effect of mianserin pretreatment on LSD induced depression of serotonergic neuron discharge activity recorded from the DRN of a freely moving cat (Day 1). The response of the *same* neuron to LSD after saline pretreatment was recorded the following day (Day 2). Pretreatments were administered 30 min prior to LSD injections and all drugs were given i.p. The data shown are standard output pulses from a variable threshold gate written out in ink on polygraph paper. Note that neither mianserin nor saline significantly affected spontaneous discharge rate of the serotonergic neuron. The effects of mianserin or saline pretreatment on LSD induced depression of firing rate were not significantly different for this cell (85.6 versus 85.0% decrease, respectively). However, this same mianserin pretreatment was completely effective in blocking the behavioral manifestations associated with this dose (50 µg/kg) of LSD (see fig. 1).

between the effects produced by these different drug treatments ($F = 25.32$, d.f. = 3,27, $P < 0.01$). Comparisons between the individual treatment groups showed that mianserin alone had no significant effect on serotonergic neuron firing rate when compared to the saline control group. None of the 7 cells tested with saline showed significant changes in discharge rate while the firing rate of

TABLE 3

Effect of mianserin pretreatment on LSD-induced depression of serotonergic unit activity. Single unit activity was recorded from serotonergic neurons within the dorsal raphe nucleus of freely moving cats. All drugs were given i.p. Post-drug firing rates were expressed as a percentage of pre-drug baseline firing rates and percent change from baseline was calculated. Post-drug firing rates were determined at a time when a unit showed a maximal change, i.e. during the peak drug effect. Individual cell responses detail, for each drug treatment, the number of neurons that exhibited significant increases or decreases in firing rate, or whose rates were unchanged. Analysis of variance revealed significant differences between drug treatments ($F_{3,27} = 25.3$, $P < 0.01$).

Drug treatment	n	% Change from baseline discharge rate (mean ± S.E.M.)	Individual cell responses		
			Increase	Decrease	No change
Saline (0.5 ml/kg)	7	−2.4 ± 3.5	0	0	7
Mianserin (1.0 mg/kg)	7	+5.4 ± 4.9	0	1	6
Saline $\xrightarrow{30'}$ LSD (50 µg/kg)	9	−66.7 ± 7.9 [a]	0	9	0
Mianserin $\xrightarrow{30'}$ LSD (50 µg/kg)	8	−53.1 ± 9.2 [b]	0	8	0

[a] Significantly different from saline and mianserin, $P < 0.05$ (Newman-Keuls).

[b] Significantly different from saline and mianserin, but *not* from saline → LSD, $P < 0.05$ (Newman-Keuls).

only 1 out of 7 cells examined was significantly altered by mianserin. On the other hand, LSD administration, after either saline or mianserin pretreatment, produced a significant decrease in discharge rate. LSD after saline pretreatment resulted in a 66.7 ± 7.9% (mean ± S.E.M.) reduction in firing rate whereas LSD after mianserin pretreatment produced a 53.1 ± 9.2% decrease in discharge rate. There was no significant difference between the magnitude of the LSD-induced depression of firing rate after mianserin pretreatment when compared to that which followed saline pretreatment. Regardless of the pretreatment condition, LSD significantly reduced the discharge rate of every cell examined. Thus, mianserin, at a dose which produced an almost complete blockade of LSD-elicited behavior, failed to alter the ability of LSD to depress serotonergic neuron discharge.

4. Discussion

We have examined the issue of whether the behavioral effects of LSD in the cat result from depression of serotonergic neurotransmission due to decreased serotonergic unit activity, the so called presynaptic hypothesis, or alternatively from an action at postsynaptic serotonin receptors. Serotonergic antagonists with a relatively selective action at postsynaptic $5HT_2$ receptors blocked the behavioral effects of LSD in a dose-dependent manner. However, these same compounds were ineffective in altering the decrease in serotonergic neuron discharge produced by LSD administration. Thus, by employing these antagonists, the effects of LSD which result from depression of serotonergic neuron discharge due to autoreceptor stimulation can be separated from those which result from an interaction of LSD at postsynaptic serotonin receptors. The data from the present study indicate that the behavioral effects of LSD in the cat are not the result of a decrease in serotonergic neuron discharge, but are dependent, at least in part, upon an action of LSD at postsynaptic serotonin receptors.

In accord with previous work (Jacobs et al., 1977b), the limb flick was the most reliable and robust of a group of emergent behaviors that are elicited by LSD administration. Recently, we and others have questioned the validity of utilizing this response, and other behaviors, as a model for the action of hallucinogenic drugs because of false positive findings with presumed non-hallucinogenic compounds such as lisuride, quipazine, apomorphine and pilocarpine (Marini et al., 1981; Marini, 1981; White et al., 1981). Although the specificity of the cat limb flick model for reflecting the action of hallucinogenic drugs is now in doubt, this model has nonetheless proven useful in examining aspects of the action of LSD and related hallucinogens, e.g. time course, dose dependence and tolerance.

The ability of serotonin receptor antagonists to block the behavioral effect of LSD was a selective action. The attenuation of the limb flick response elicited by LSD was not due to sedation or a general impairment of motor ability since effective doses of the antagonists produced no evidence of sedation nor did they decrease locomotor activity. In fact, administration of the antagonists tended to activate the animals as reflected in higher locomotor scores. The inability of the serotonergic antagonists to block limb flicking behavior produced by apomorphine or lisuride injection is further evidence for their selective action in reducing LSD-induced behavior. Interestingly, the serotonergic antagonists also blocked the limb flick response elicited by DOM, which unlike the indole nucleus class of hallucinogen represented by LSD, is a phenylethylamine derivative hallucinogen. The ability of the serotonergic antagonists to block limb flicking elicited by hallucinogens representing two different classes, but not the same behavior produced by the non-hallucinogens apomorphine, and lisuride, suggests that serotonin receptor blockade specifically interrupts the behavioral manifestations of hallucinogenic compounds. This is consistent with recent evidence that in addition to LSD, DOM has direct serotonin agonist properties (Sloviter et al., 1980; Glennon et al., 1983).

A relationship between serotonin receptors and hallucinogenic drug action has also been demonstrated by a large number of experiments utilizing several other behavioral paradigms. In operant experiments the response suppressant effects produced by a number of hallucinogenic compounds

are blocked by prior administration of serotonin receptor antagonists, but not by pretreatment with dopaminergic antagonists (Commissaris et al., 1980; 1981; Dwoskin and Sparber, 1983). Conversely, the same behavioral model has shown that the response suppressant effects of non-hallucinogens such as amphetamine and phenobarbital cannot be blocked by serotonergic antagonists (Commissaris et al., 1981). Moreover, and critical for the present argument, it seems unlikely that the attenuation of the limb flick response by serotonergic antagonists can be simply dismissed as a blockade of a motor response peculiar to hallucinogens since a similar blocking effect has been observed in a large number of studies in which the hallucinogen acted as a discriminative stimulus (Browne and Ho, 1975; Winter, 1975; 1978; Kuhn et al., 1978; Silverman and Ho, 1980). Such paradigms are presumed to reflect actions of hallucinogenic compounds at higher levels of CNS integration.

LSD has been shown to bind to $5HT_1$ and $5HT_2$ receptors with near equal affinity (Peroutka and Snyder, 1979). In addition, although an interaction between LSD and serotonin autoreceptors is difficult to demonstrate with binding techniques, electrophysiological experiments in which LSD mimics the effect of serotonin on serotonergic neurons suggests that LSD does act upon serotonin autoreceptors, perhaps with even greater affinity than for postsynaptic $5HT_1$ and $5HT_2$ sites (Haigler and Aghajanian, 1974a). Mianserin, ketanserin and metergoline are serotonin receptor antagonists that have been shown to possess relatively high selectivity ratios that favor binding to $5HT_2$ versus $5HT_1$, receptor sites (Leysen et al., 1981). Our electrophysiological data indicate that mianserin does not alter the spontaneous activity of serotonergic neurons within the DRN nor does it alter the ability of LSD to inhibit serotonergic unit activity. These findings are consistent with previous work conducted in chloral hydrate anesthetized rats which demonstrated that classical serotonergic antagonists are inactive at serotonin autoreceptors (Haigler and Aghajanian, 1974b). Taken together, the receptor binding and electrophysiological data suggest that the serotonergic antagonists that we have employed are strongly preferential for blocking the action of LSD at $5HT_2$ receptor sites with little or no effect on LSD's interaction with the $5HT_1$ site or serotonin autoreceptors.

Binding studies also have revealed that LSD acts at receptor sites that are not serotonergic (Leysen et al., 1981). In similar fashion, the three serotonergic antagonists employed in the present study exert actions at receptor sites that are not serotonergic (Leysen et al., 1981). However, while high affinity binding to $5HT_2$ receptors is consistent among these drugs, their binding affinity characteristics at other receptor sites is not homogeneous. Thus, although LSD and the serotonergic antagonists can act upon other receptors, their shared affinity for $5HT_2$ sites strongly suggests that the attenuation of LSD-induced behavior by prior administration of serotonergic antagonists is specifically due to a blockade of $5HT_2$ receptors. This does not imply, however, that all of the psychological and behavioral effects of LSD and related hallucinogens can be accounted for by an action at postsynaptic serotonergic receptors. Nor does this imply that the action of these hallucinogens is a purely agonist action at the serotonergic receptor. An agonist action at postsynaptic serotonergic receptors may be *necessary* for hallucinogenic activity, but other serotonergic and non-serotonergic actions may modulate and interact with this effect.

Two other findings from our laboratory, which add generality to the present results, deserve brief mention. We have observed similar results to the present electrophysiological ones for serotonergic neurons recorded from the nucleus centralis superior (NCS) in freely moving cats, and we have found ketanserin to be ineffective for blocking LSD's effect on DRN or NCS serotonergic neurons (Rasmussen, Heym and Jacobs, unpublished observations).

We have demonstrated that serotonergic antagonists with an ability for blocking $5HT_2$ receptors can block the behavioral effects of LSD without altering its presynaptic action on serotonergic neurons. This finding is in accord with results from a large number of studies employing other animal behavioral models where the behavioral effects of LSD are reduced by

serotonergic antagonists. Thus, the action of LSD at postsynaptic receptor sites appears to be a critical factor for the manifestation of its behavioral effects. The concomitant decrease in serotonergic neuron discharge, representing LSD's presynaptic effect, is apparently not of primary importance for LSD induced alterations of behavior. These findings may take on added significance if they can be extended to include the psychoactive effects of LSD. In this context, it is interesting to recall reports (Resnick et al., 1964; Grof and Dytrych, 1965) that have cited a decrease in the hallucinogenic potency of LSD in humans following pharmacological treatments known to alter postsynaptic serotonin receptor density in animal studies.

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References

Appel, J.B., J.A. Joseph, E. Utsey and L.L. Hernandez, 1977, Sensitivity to psychoactive drugs and the serotonergic neuronal system, Commun. Psychopharmacol. 1, 541.

Bennett, J.L. and G.K. Aghajanian, 1974, D-LSD binding to brain homogenates: possible relationship to serotonin receptors, Life Sci. 15, 1935.

Bennett, J.P. and S.H. Snyder, 1976, Serotonin and lysergic acid diethylamide binding in rat brain membranes: relationship to postsynaptic serotonin receptors, Mol. Pharmacol. 12, 373.

Beretta, C., R. Ferrini and A.H. Glasser, 1965, 1,6-Dimethyl-8β-carbobenzyloxy-aminomethyl-10α-ergoline, a potent and long-lasting 5-hydroxytryptamine antagonist, Nature 207, 421.

Browne, R.G. and B.T. Ho, 1975, Role of serotonin in the discriminative stimulus properties of mescaline, Pharmacol. Biochem. Behav. 3, 429.

Commissaris, R.L., W.H. Lyness, K.E. Moore and R.H. Rech, 1981, Differential antagonism by metergoline of the behavioral effects of indolealkylamine and phenethylamine hallucinogens in the rat, J. Pharmacol. Exp. Ther. 219, 170.

Commissaris, R.L., D.R. Semeyn, K.E. Moore and R.H. Rech, 1980, The effects of 2,5-dimethoxy-4-methylamphetamine (DOM) on operant behavior: interactions with other neuroactive agents, Commun. Psychopharmacol. 4, 393.

Dwoskin, L.P. and S.B. Sparber, 1983, Behaviorally inactive doses of mianserin antagonize the suppressant effect of lysergic acid diethylamide on a fixed-ratio operant, J. Pharmacol. Exp. Ther. 225, 77.

Ferrini, R. and A.H. Glasser, 1965, Antagonism of central effects of tryptamine and 5-hydroxytryptophan by 1,6-dimethyl-8β-carbobenzyloxyaminomethyl-10α-ergoline. Interference with amphetamine and DOPA, Psychopharmacologia 8, 271.

Freedman, D.X., 1961, Effects of LSD-25 on brain serotonin, J. Pharmacol. Exp. Ther. 134, 160.

Gaddum, J.H. and K.A. Hameed, 1954, Drugs which antagonize 5-hydroxytryptamine, Br. J. Pharmacol. 9, 240.

Glennon, R.A., R. Young, J.M. Jacyno, M. Slusher and J.A. Rosecrans, 1983, DOM-stimulus generalization to LSD and other hallucinogenic indolealkylamines, European J. Pharmacol. 86, 453.

Grof, S. and Z. Dytrych, 1965, Blocking of LSD reaction by premedication with Niamid, Activ. Nerv. Super. 7, 306.

Haigler, H.J. and G.K. Aghajanian, 1974a, Lysergic acid diethylamide and serotonin: A comparison of effects on serotonergic neurons and neurons receiving serotonergic input, J. Pharmacol. Exp. Ther. 188, 688.

Haigler, H.J. and G.K. Aghajanian, 1974b, Peripheral serotonin antagonists: failure to antagonize serotonin in brain areas receiving a priminent serotonergic input, J. Neural Transm. 35, 257.

Jacobs, B.L., 1983, Mechanism of action of hallucinogenic drugs: Focus upon postsynaptic serotonergic receptors, in: Psychopharmacology, eds. D.G. Grahame-Smith and P.J. Cowen, Elsevier Biomedical Press, Amsterdam, p. 344.

Jacobs, B.L., J. Heym and K. Rasmussen, 1982a, Action of hallucinogenic drugs at postsynaptic serotonergic receptors, Psychopharmacol. Bull. 18, 168.

Jacobs, B.L., J. Heym and M.E. Trulson, 1981, Cats develop tolerance to d-amphetamine's effects upon locomotion and stereotyped behavior, European J. Pharmacol. 69, 353.

Jacobs, B.L., K. Rasmussen and J. Heym, 1982b, Evidence that some of the behavioral effects of LSD are mediated by a direct action at postsynaptic serotonergic receptors, Soc. Neurosci. Abstr. 8, 390.

Jacobs, B.L. and M.E. Trulson, 1981, The role of serotonin in the action of hallucinogenic drugs, in: Serotonin Neurotransmission and Behavior, ed. B.L. Jacobs and A. Gelperin (MIT Press, Cambridge, MA) p. 366.

Jacobs, B.L., M.E. Trulson, A.D. Stark and G.R. Christoph, 1977a, Comparative effects of hallucinogenic drugs on behavior of the cat, Commun. Psychopharmacol. 1, 243.

Jacobs, B.L., M.E. Trulson and W.C. Stern, 1977b, Behavioral effects of LSD in the cat: Proposal of an animal behavior model for studying the actions of hallucinogenic drugs, Brain Res. 132, 301.

Kuhn, D.M., F.J. White and J.B. Appel, 1978, The discriminative stimulus properties of LSD: Mechanisms of action, Neuropharmacology 17, 257.

Leysen, J.E., F. Awouters, L. Kennis, P.M. Laudron, J. Vandenberk and P.A.J. Janssen, 1981, Receptor binding profile of R 41468, a novel antagonist at $5HT_2$ receptors, Life Sci. 28, 1015.

Lucki, I. and A. Frazer, 1982, Prevention of the serotonin syndrome in rats by repeated administration of monoamine oxidase inhibitors but not tricyclic antidepressants, Psychopharmacology 77, 205.

Marini, J.L., 1981, Pilocarpine, a non-hallucinogenic cholinergic agonist, elicits limb flicking in cats, Pharmacol. Biochem. Behav. 15, 865.

Marini, J.L., B.L. Jacobs, M.H. Sheard and M.E. Trulson, 1981, Activity of a non-hallucinogenic ergoline derivative, lisuride, in an animal behavior model for hallucinogens, Psychopharmacology 73, 328.

Peroutka, S.J. and S.H. Snyder, 1979, Multiple serotonin receptors: Differential binding of [^{3}H]spiroperidol, Mol. Pharmacol. 16, 687.

Peroutka, S.J. and S.H. Snyder, 1980, Long-term antidepressant treatment decreases spiroperidol-labeled serotonin receptor binding, Science 210, 88.

Peroutka, S.J. and S.H. Snyder, 1981, [^{3}H]Mianserin: Differential labeling of serotonin$_2$ and histamine$_1$ receptors in rat brain, J. Pharmacol. Exp. Ther. 216, 142.

Resnick, O., D.M. Krus and M. Raskin, 1964, LSD-25 action in normal subjects treated with a monoamine oxidase inhibitor, Life Sci. 3, 1207.

Resnick, O., D.M. Krus and M. Raskin, 1965, Accentuation of the psychological effects of LSD-25 in normal subjects treated with reserpine, Life. Sci. 4, 433.

Savage, D.D., J. Mendels and A. Frazer, 1980, Monoamine oxidase inhibitors and serotonin uptake inhibitors: Differential effects on [^{3}H]serotonin binding sites in rat brain, J. Pharmacol. Exp. Ther. 212, 259.

Silverman, P.B. and B.T. Ho, 1980, The discriminative stimulus properties of 2,5-dimethoxy-4-methylamphetamine (DOM): differentiation from amphetamine, Psychopharmacology 68, 209.

Sloviter, R.S., E.G. Drust, D.P. Damiano and J.D. Connor, 1980, A common mechanism for lyergic acid, indolealkylamine and phenethylamine hallucinogens: Serotonergic mediation of behavioral effects in rats, J. Pharmacol. Exp. Ther. 214, 231.

Snider, R.S. and W.T. Niemer, 1961, A Stereotaxic Atlas of the Cat Brain (University of Chicago Press).

Trulson, M.E., J. Heym and B.L. Jacobs, 1981, Dissociations between the effects of hallucinogenic drugs on behavior and raphe unit activity in freely moving cats, Brain Res. 215, 275.

Trulson, M.E. and B.L. Jacobs, 1979, Alterations of serotonin and LSD receptor binding following repeated administration of LSD, Life Sci. 24, 2053.

Trulson, M.E., C.A. Ross and B.L. Jacobs, 1976, Behavioral evidence for the stimulation of CNS serotonin receptors by high doses of LSD, Psychopharmacol. Commun. 2, 149.

Trulson, M.E., C.A. Ross and B.L. Jacobs, 1977, Lack of tolerance to the depression of raphe unit activity by lysergic acid diethylamide, Neuropharmacology 16, 771.

White, F.J., A.M. Holohean and J.B. Appel, 1981, Lack of specificity of an animal behavior model for hallucinogenic drug action, Pharmacol. Biochem. Behav. 14, 339.

Winter, J.C., 1975, Blockade of the stimulus properties of mescaline by a serotonin antagonist, Arch. Int. Pharmacodyn. Ther. 214, 250.

Winter, J.C., 1978, Stimulus properties of phenethylamine hallucingens and lysergic acid diethylamide: The role of 5-hydroxytryptamine, J. Pharmacol. Exp. Ther. 204, 416.

Wooley, D.W. and E. Shaw, 1954, A biochemical and pharmacological suggestion about certain mental disorders, Proc. Nat. Acad. Sci. U.S.A. 40, 228.