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ANTAGONISM OF THE LSD CUE BY PUTATIVE SEROTONIN ANTAGONISTS: RELATIONSHIP TO INHIBITION OF IN VIVO [^{3}H]SPIROPERIDOL BINDING

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In two groups of rats trained to discriminate 0.08 or 0.16 mg/kg of lysergic acid diethylamide (LSD) from saline, pirenperone and ketanserin completely blocked the stimulus effect of LSD. Pizotifen (BC-105) blocked the LSD cue when the training dose was 0.08 mg/kg, but had variable effects in the 0.16 mg/kg of LSD-trained group. The antagonism of the 0.08 mg/kg cue occurred at doses of the antagonists which blocked [^{3}H]spiroperidol labeled 5-HT$_2$ receptors in the frontal cortex in vivo; binding in the striatum was unaffected by the LSD antagonists. However, in doses which produce the LSD cue, neither LSD nor the 5-HT agonist, 5-methoxy-N,N-dimethyltryptamine, which substitutes for LSD, inhibited the binding in either the cortex or the striatum. The results are discussed in relation to the possible neuropharmacological basis for the LSD cue.

INTRODUCTION

It is now well established that many of the behavioral, biochemical and electrophysiological effects of lysergic acid diethylamide (LSD) and related 'hallucinogens' are mediated by 5-hydroxytryptamine (5-HT) receptors[8]. Important support for this contention comes from findings that LSD: (1) decreases the firing rate of 5-HT containing neurons in the midbrain raphe nuclei[1]; (2) displaces putative 5-HT receptor radioligands from neuronal membrane preparations[22]; and (3) has both discriminative and rate-depressing effects that are blocked specifically by 5-HT antagonists[3,23].

However, the precise neuropharmacological actions of LSD are still unclear – at least those that underlie hallucinogenic aspects of the drug. Thus, non-hallucinogenic agents such as lisuride produce effects that also occur following LSD, including similar, though not identical, cues[25] and inhibition of the firing rate of raphe 5-HT neurons[24]; moreover, drugs which are hallucinogenic, such as the phenylethylamine mescaline, do not inhibit the raphe (e.g. ref. 18). Evidence that 5-HT receptors are heterogenous suggests a possible basis for clarification of the effects of at least some serotonergic drugs. One receptor subtype (5-HT$_2$) is labeled by [^{3}H]spiroperidol in the frontal cortex, and further, antagonist displacement of the radioligand correlates highly with inhibition of an excitatory 5-HT effect, i.e. the head-twitch response in rodents[22]. Recent research with selective 5-HT$_2$ antagonists has fur-

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ther implicated these receptors in mediating head-twitches and related effects (e.g. the 'serotonin syndrome') induced by relatively high doses of certain serotonergic drugs[6,9,20]. However, the possibly differential role(s) of 5-HT receptor subtypes in behavioral effects of LSD at higher levels of complexity have been relatively unexplored. While pirenperone and ketanserin, two newly synthesized compounds with high affinities for 5-HT_2 receptors[12,17], completely and selectively antagonize the discriminative stimulus properties of 0.16 mg/kg LSD in the rat[5], these compounds also interact with other neurotransmitter systems, notably dopamine and histamine (pirenperone), and norepinephrine (ketanserin)[12,20].

The present experiments were designed to obtain more direct evidence for the involvement of 5-HT_2 receptors in the effects of LSD. This was accomplished by comparing the ability of the putative 5-HT_2 antagonists pirenperone, ketanserin and of pizotifen to block the discriminative stimulus effects of LSD with the abilities of the same doses of these agents to occupy 5-HT_2 receptors in rat frontal cortex in vivo. In addition, a comparison was made of the abilities of LSD and the 5-HT agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT), which substitutes for LSD, to occupy 5-HT_2 receptors. Finally, since dopamine (DA) has sometimes been implicated in the behavioral effect of LSD, e.g. ref. 8, the effect of these treatments upon binding of [^{3}H]spiroperidol to presumed (DA) receptor in the striatum was also studied.

MATERIALS AND METHODS

Male Sprague–Dawley albino rats were used in all experiments. The animals were housed individually in a room of constant temperature (20–21 °C) and relative humidity (40–60%). Room lights were on from 07.00 to 19.00 h; food and water were freely available except as indicated (below).

Drug discrimination procedure

Details of the behavioral procedure have been described elsewhere[4]. Eight operant chambers (BRS/LVE type 143-24) equipped with two levers and a dipper (0.1 ml) were used. Animals (n = 16) weighing 300–350 g were deprived to 80–85% of their free-feeding body weights, by restricting water intake to that received in the chamber, and trained to respond under a fixed ratio (FR) schedule of 20 responses for water reinforcement. Fifteen min following i.p. injection of 0.08 mg/kg LSD (n = 8), responding was reinforced on a designated (drug) lever; responding on the other lever had no programmed consequences. Following an i.p. saline injection, responding was reinforced on the lever opposite to the drug lever. A second group of animals (n = 8) was trained similarly to discriminate 0.16 mg/kg of LSD from saline. Discrimination accuracy was expressed as the percent correct responses (appropriate to injection of LSD or saline) prior to the delivery of the first reinforcer (i.e. 20 × 100/(20 + number of incorrect responses)).

Following the acquisition of stable, high (>90%) discrimination accuracy for at least 1 week, test sessions were conducted every 2–4 days. In substitution tests, the abilities of other doses of LSD or of novel drugs, to mimic the training dose of LSD were determined; in combination (antagonism) tests, various drugs were given prior to LSD to ascertain the degree of attenuation of drug (LSD) lever responding. Test sessions were terminated after completion of the 20 responses (= 1 FR) on either lever or, when a maximum of 20 min had elapsed. Only data from animals that made at least 10 responses and from sessions during which at least half of the animals responded were used.

[^{3}H]spiroperidol binding assay

Rats weighing 150–170 g were anesthetized locally by injection of 0.1 ml of 5% lidocaine s.c. above the femoral vein. A small incision was made to expose the vein into which was injected 5 μg/kg (288 μCi/kg) of [^{3}H]spiroperidol (New England Nuclear, Boston, MA; spec. act. 22.8 Ci/mMol) in a volume of 2 μl/g b. wt. Following the administration of putative receptor binding inhibitors (below; n = 2–3 per dose), all animals were killed by decapitation 2 h after the administration of [^{3}H]spiroperidol. The brain was removed rapidly and the frontal one-third of the

cortex (192 ± 4 mg), striatum (79 ± 2 mg), and the frontal half of the cerebellum (116 ± 3 mg) (means ± S.E.M.) were dissected. The tissue was weighed and homogenized in 200 vols. of cold Tris citrate buffer (20 mMol, pH 7.4) using an Ultra Turrax homogenizer (10 s at 3/4 speed). Duplicate aliquots of 1 ml (corresponding to 5 mg of original tissue) were filtered through Whatman GF/C glass fiber filters, which were then washed twice with 5 ml cold buffer. The radioactivity trapped on the filters was extracted overnight and counted using conventional liquid scintillation techniques. The data are expressed as dpm/mg wet tissue with no further corrections. The levels of unspecific binding (see Results) were subtracted from levels of total binding to obtain a measure of specific binding (expressed as a percentage of control).

Drugs

The following drugs were dissolved in deionized (DI) water, except when otherwise indicated, and injected in a volume of 1 ml/kg: (+)-butaclamol HCl (Research Biochemicals, Wayland, MA) in ethanol diluted with DI water, s.c., 150 min before test; haloperidol (Janssen Pharmaceutica, Beerse, Belgium) diluted with saline, from ampoules, s.c., 60 min before test; ketanserin bitartrate (Janssen) i.p., 60 min before test; D-LSD bitartrate (National Institute on Drug Abuse, Research Triangle, NC) in saline, i.p., 15 min before test; pirenperone (Janssen) in DI water with a few drops of HCl, i.p., 60 min before test; pizotifen maleate (BC-105; Sandoz, East Hanover, NJ), i.p., 60 min before test. Saline (0.9%) was administered i.p. as a vehicle control. Injection intervals were identical in the drug discrimination and binding experiments.

Statistical analysis

The dose–effect curves were analyzed by log-probit methods using the PROBIT programme (SAS Institute, Cary, NC, U.S.A.), which generated ED_{50} and ID_{50} values; i.e. doses producing 50% LSD appropriate responding, or inhibiting the cue to 50%, respectively. The binding data were evaluated with *t*-tests for independent groups.

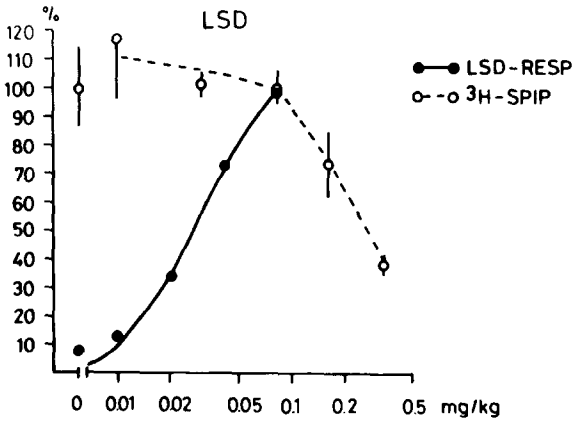

Fig. 1. Dose–response curve of LSD in animals (n = 8) trained to discriminate the stimulus properties of 0.08 mg/kg of LSD from saline (solid line and symbols). The effect of saline (alone) is indicated at 0 mg/kg. Also shown is the ability of LSD to inhibit specific in vivo [^{3}H]spiroperidol binding in the frontal cortex (dotted line, open symbols; means ± S.E.M. or range (n = 2–3 per dose)). The level of specific binding in saline (control) animals is indicated at 0 mg/kg.

RESULTS

The dose–response curve of the discriminative stimulus effects of different test doses of LSD in animals trained with 0.08 (Fig. 1) and 0.16 mg/kg (data not shown) of LSD, were orderly and monotonic; ED_{50}s were 0.024 and 0.042 mg/kg, respectively.

In the binding experiments, 5 mg/kg of (+)-butaclamol displaced 80–90% of the total [^{3}H]spiroperidol in the frontal cortex and striatum (Table I); the remaining levels of binding

TABLE I

[^{3}H]Spiroperidol binding following i.v. injection of 5 μg/kg

[^{3}H]Spiroperidol was injected 2 h before decapitation. Butaclamol was injected s.c. 30 min before [^{3}H]spiroperidol. n = 3 per group. Values denote means ± S.E.M.

	Radioactivity (dpm/mg wet tissue)		
	Frontal cortex	*Striatum*	*Cerebellum*
Total binding	137 ± 16	494 ± 27	42 ± 1
(+)-Butaclamol 5 mg/kg	26 ± 3	56 ± 3	18 ± 2
Specific binding	111 ± 16	438 ± 27	27 ± 1
Percent of total	81 ± 14%	89 ± 6%	43 ± 4%

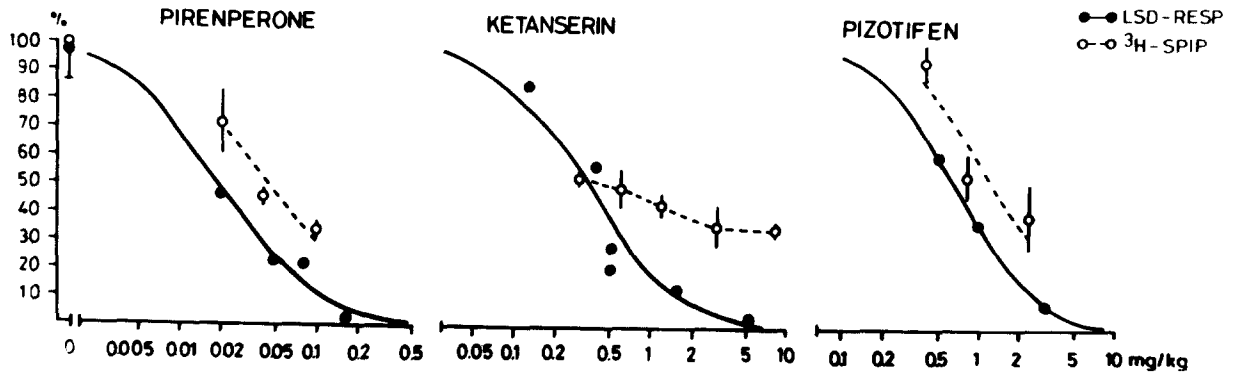

Fig. 2. Abilities of pirenperone, ketanserin and pizotifen (i.p.; t = 60 min) to antagonize the stimulus properties of 0.08 mg kg of LSD in animals (n = 8) trained to discriminate this dose of LSD from saline (solid line); the effect of the LSD training dose (alone) is indicated at 0 mg/kg. Also shown is the inhibitory effect of pirenperone, ketanserin and pizotifen (i.p.; t = 60 min) on specific in vivo [^{3}H]spiroperidol binding in the frontal cortex (dotted lines, open symbols; means ± S.E.M. or range (n = 2–3 per dose)). The level of specific binding in saline-treated (control) animals is indicated at 0 mg kg.

in these areas were defined as unspecific binding (this was roughly similar to the cerebellar level, which is often used as an alternative measure of unspecific binding). Doses of LSD up to 0.08 mg/kg did not affect binding; higher doses did inhibit binding in the frontal cortex (Fig. 1), but not in the striatum (not shown). A single dose of 5-MeODMT (4 mg/kg), which has been found previously to substitute completely for 0.08 mg/kg of LSD[26], did not affect [^{3}H]spiroperidol binding in either the frontal cortex or the striatum (97% ± 11, $t = 0.14$, df = 3, n.s. and 86% ± 4, $t = 2.44$, df = 3, n.s., respectively, compared to control).

As can be seen in Fig. 2, pirenperone and pizotifen blocked the stimulus properties of 0.08 mg/kg of LSD (ID_{50}s were 0.02 and 0.7 mg/kg, respectively); these effects occurred at doses of the antagonists that also inhibited [^{3}H]spiroperidol binding in the frontal cortex (IC_{50}s were 0.044 and 1.47 mg/kg, respectively); levels of binding in the striatum were unaffected (data not shown). Ketanserin (Fig. 2) also blocked the behavioral effect of 0.08 mg/kg LSD (ID_{50} was 0.34 mg/kg). However, within the cue-antagonistic range of doses, the effect of ketanserin on [^{3}H]spiroperidol binding in the frontal cortex was shallow; that is, a ceiling was reached at 30–40% of control values (IC_{50} was 0.52 mg/kg). No dose of ketanserin affected striatal binding.

A relatively high dose of haloperidol (0.31 mg/kg), which did not alter the stimulus effect of 0.08 mg/kg of LSD (88% LSD responses), affected marginally [^{3}H]spiroperidol binding in the striatum (80% of control; $t = 3.1$, df = 4, $P < 0.05$) but had no effect of binding in the frontal cortex (88% of control; $t = 0.53$, df = 4, n.s.).

The effects of pirenperone and ketanserin on the LSD cue in animals trained on 0.16 mg/kg were generally similar to those obtained in animals trained on 0.08 mg/kg, although the ID_{50} values were higher (0.037 and 1.48 mg/kg, respectively). On the other hand, pizotifen had highly variable effects in rats trained to discriminate 0.16 mg/kg of LSD from saline; in fact, the 'dose–response' curve was U-shaped, with maximal antagonism effect around 1–2 mg/kg (data not shown).

DISCUSSION

The present data confirm and extend previous findings that 5-HT antagonists, including pirenperone, pizotifen and ketanserin, block the discriminative stimulus properties of relatively low doses of LSD[5,14]. A common action of these compounds is to inhibit [^{3}H]spiroperidol binding to putative 5-HT_2 receptors in the frontal cortex (refs. 12, 20 and present data). Since at least ketanserin and pirenperone have low affinity for 5-HT_1 sites[15,16], these observations might imply that the stimulus effect of LSD involves 5-HT_2

(primarily or exclusively) receptor antagonism. This is supported further by the finding (above) that antagonism of the 0.08 mg/kg cue occurs at doses of the 5-HT antagonists which also occupy in vivo 5-HT_2 receptors. However, it is noteworthy that ketanserin, which antagonized completely the stimulus effect of LSD, inhibited [^{3}H]spiroperidol binding to an asymptotic level of about 35%. Such a 'shallowing' may indicate that [^{3}H]spiroperidol binding sites are heterogeneous[11,19], and that ketanserin binds to a subset (i.e. 5-HT_2 receptors) of all the sites that are labeled by [^{3}H]spiroperidol.

It is interesting that pizotifen did not antagonize the 0.16 mg/kg LSD cue in an orderly, dose-dependent fashion. However, it has been noted previously that relatively high doses of some 5-HT antagonists (including pizotifen) also have LSD-like properties in DD situations[5]. Therefore, the present finding with pizotifen (when given in combination with LSD) may have been the result of mixed agonist/antagonist effects at LSD binding sites.

The rat frontal cortex receives a relatively dense, but diffuse innervation of serotonergic fibers which originate in the dorsal raphe nucleus[2]. The present finding of a relationship between blockade of cortical 5-HT_2 receptors and antagonism of LSD's stimulus effect, suggests that cortical raphe projections are involved (somehow) in the LSD cue (see also ref. 10). This is supported by the findings that very low doses of LSD and other hallucinogens suppress the firing rate of 5-HT raphe cells, probably by an action on 5-HT autoreceptors[1] and, that no tolerance develops to this effect or to the stimulus effects of LSD.

LSD, and the direct 5-HT agonist 5-MeODMT, did not affect [^{3}H]spiroperidol binding within the range of doses which produce the LSD cue. The relative inability of these drugs to affect [^{3}H]spiroperidol binding may be taken to indicate that LSD and 5-MeODMT have high efficacy at these sites. That drugs sometimes have potent effects in doses which occupy only a small fraction of their relevant receptors is not unusual (e.g. ref. 13). Alternatively, it is also possible that the stimulus effects of LSD are mediated by actions at 5-HT_1 as well as 5-HT_2 receptor sites[7], since this drug has high affinity for putative 5-HT_1 receptors labeled by [^{3}H]5-HT[22]. However, so-called 5-HT_1 sites probably comprise multiple receptor subtypes[19,21] and, until further receptor binding data are available, it is premature to speculate on the existence of a particular subtype at which LSD might produce its discriminative effects.

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