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ORIGINAL INVESTIGATION

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The role of the 5-HT_{2A} and 5-HT_{2C} receptors in the stimulus effects of hallucinogenic drugs I: Antagonist correlation analysis

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Abstract Investigations conducted over the past 3 decades have demonstrated that serotonergic receptors, specifically the 5-HT_{2A} and 5-HT_{2C} subtypes, play an important role in the behavioral effects of hallucinogenic compounds. The present study was designed to determine the respective significance of these two receptors in the stimulus effects of LSD and (−)DOM in the rat. Specifically, the interactions of a series of serotonergic antagonists (risperidone, pirenpirone, metergoline, ketanserin, loxapine, LY53857, pizotyline, spiperone, cyproheptadine, mesulergine, promethazine, and thioridazine) with the LSD stimulus and the (−)DOM stimulus in LSD-trained subjects was defined. From these data, IC_{50} values were determined for the inhibition of the LSD-appropriate responding elicited by either 0.1 mg/kg LSD (15-min pretreatment time) or 0.4 mg/kg (−)DOM (75-min pretreatment). In addition, the affinities of these antagonists for 5-HT_{2A} and 5-HT_{2C} receptors were determined in radioligand competition studies. 5-HT_{2A} affinity correlated significantly with IC_{50} values for the blockade of the LSD ($r = +0.75$, $P < 0.05$) and (−) DOM ($r = +0.95$, $P < 0.001$) stimuli in the LSD trained subjects. 5-HT_{2C} affinity did not correlate significantly with either series of IC_{50} values. These data indicate that (1) the stimulus effects of LSD, and (2) the substitution of (−)DOM for the LSD stimulus are mediated by agonist activity at 5-HT_{2A} receptors.

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Introduction

Drug-induced stimulus control has been applied to the study of hallucinogens for more than 20 years (for recent reviews see Glennon 1994; Winter 1994). The drugs most often chosen to serve as training stimuli have been the indolamine, lysergic acid diethylamide (LSD) (Hirschhorn and Winter 1971) and the phenylalkylamine, 2,5,-dimethoxy-4-methylamphetamine (DOM) (Silverman and Ho 1978). The subjective effects of DOM and LSD in humans have been extensively investigated, and their status as human hallucinogens is generally accepted (Shulgin et al. 1973; Shulgin and Shulgin 1992). Evidence accumulated over the past 2 decades has indicated that the stimulus effects of hallucinogens are mediated by interactions with serotonin (5-hydroxytryptamine, 5-HT) receptors (Appel et al. 1982; Glennon et al. 1982, 1984b; Winter 1978).

Biochemical studies have demonstrated that the pharmacology of hallucinogens is complex. This is particularly true of LSD, which has been reported to bind with nanomolar affinity to serotonergic receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A} and 5-HT_{2C}), dopaminergic receptors (D_2), and adrenergic receptors (alpha-1 and 2) (Burt et al. 1976; Creese et al. 1976; U'Prichard et al. 1977; Meibach et al. 1980; Leysen 1985; Hoyer 1988; Leysen et al. 1988). The binding profile of DOM is much more selective than that of LSD. DOM has been reported to bind with only micromolar affinity to 5-HT_{1A}, 5-HT_{1B}, beta-1, alpha-1 and 2 adrenergic, histaminergic and dopaminergic (D_2) receptors (Leysen et al. 1989). Both LSI and DOM possess nanomolar affinity for both 5-HT_{2A} and 5-HT_{2C} receptors

(Glennon et al. 1984a; Titeler et al. 1988). With respect to the stimulation of phosphoinositide (PI) hydrolysis, these compounds have been described as partial agonists at 5-HT_2 receptors (Sanders-Bush et al. 1988). In an agonist correlation analysis study, the EC_{50} values for a series of hallucinogens to substitute for the stimulus effects of DOM were reported to correlate with their affinities for both 5-HT_{2A} and 5-HT_{2C} receptors (Titeler et al. 1988). In addition, antagonists of 5-HT_{2A} and 5-HT_{2C} receptors have been found to block the stimulus effects of hallucinogens (Winter 1975, 1978; for review see Appel and Cunningham 1986; Cunningham and Appel 1987).

While these previous studies indicate that hallucinogen-induced stimulus control is mediated by agonist interactions with central 5-HT_2 receptors, they fail to differentiate the relative roles of the 5-HT_{2A} and 5-HT_{2C} receptor subtypes. This problem can be attributed to the lack of selective compounds for either receptor. A high degree of correlation ($r = 0.83$) between 5-HT_{2A} and 5-HT_{2C} receptor affinity was described for the series of hallucinogens used in the agonist correlation analysis of Titeler et al. (1988), with no agent possessing even one order of magnitude higher affinity for either receptor (Glennon et al. 1992a,b). With respect to the serotonergic antagonists, few compounds have been identified which possess greater than one order of magnitude higher affinity for either receptor (Sanders-Bush and Breeding 1988; Leysen 1990; Wijngaarden et al. 1990; Glennon and Dukat 1991; Sahin-Erdemli et al. 1991). Of the antagonists described as possessing some selectivity within the 5-HT_2 receptor system, all have been shown to possess greater affinity for 5-HT_{2A} receptors than 5-HT_{2C} receptors. Applications of these compounds in drug discrimination studies have yielded inconsistent results. Spiperone, an antagonist with more than 2600 times greater affinity for the 5-HT_{2A} receptor than the 5-HT_{2C} receptor (Fiorella et al. 1994a), has been reported to be ineffective in blocking the stimulus effects of LSD (Appel and Cunningham 1986). In contrast, a structural analog of spiperone with similar 5-HT_{2A} selectivity was reported to block the DOM stimulus (Ismaiel et al. 1993). In addition, ketanserin and pirenpirone, compounds with approximately 30 times greater potency at the 5-HT_{2A} site (Fiorella et al. 1995a), have been reported to antagonize fully the stimulus effects of hallucinogens (Colpaert et al. 1982; Nielsen et al. 1985; Appel et al. 1986).

Biochemical and behavioral observations indicate several important considerations for the application of stimulus control to the study of hallucinogens. First, due to their pharmacological complexity, the mechanism by which hallucinogens elicit the interoceptive state governing stimulus control in animal subjects may not be equivalent to the mechanism by which those same agents elicit hallucinations in humans. Second, due to their pharmacological dissimilarities, the mechanistic basis for the interoceptive states created by different hallucinogens in animal subjects may differ significantly. Correspondingly, conclusions based on data describing the stimulus effects of a single hallucinogenic training drug are necessarily biased toward the particular idiosyncrasies of that training compound, and therefore may not be expected to apply to hallucinogens in general.

The simultaneous study of multiple hallucinogenic compounds is one method by which possibly to overcome these potential shortcomings of the drug discrimination paradigm. Focusing on the common characteristics of the discriminative stimuli created by hallucinogens increases the probability of defining mechanisms which are relevant to their common effect of hallucinogenesis, while simultaneously de-emphasizing the idiosyncratic characteristics of each stimulus. Specifically, this can be accomplished by determining the mechanism by which the stimulus properties of one hallucinogen substitute for those of another.

The present study was designed to differentiate the respective roles of the 5-HT_{2A} and 5-HT_{2C} receptors in the stimulus effects of hallucinogenic compounds. The method of antagonist correlation analysis (Friedman et al. 1984; Fiorella et al. 1995b) was employed to determine the pharmacological basis for (1) LSD-induced stimulus control (2) the substitution of (−)DOM for the LSD stimulus.

Materials and methods

Animals

Forty-five Male Fischer 344 rats were obtained from Harlan Sprague-Dawley Inc. (Indianapolis, Ind.). They were housed in pairs under a natural light-dark cycle and allowed free access to water in the home cage. Subjects were fed immediately following experimental sessions. Caloric intake was controlled to yield a mean body weight of 250 g.

Apparatus

Six small animal test chambers (Coulbourn Instruments Model E10-10) housed in larger light-proof, sound insulated boxes were used for all experiments. Each box had a house light and exhaust fan. The chamber contained two levers mounted on opposite ends of one wall. Centered between the levers was a dipper that delivered 0.1 ml sweetened condensed milk diluted 2:1 with tap water.

Drug-induced stimulus control procedure

Subjects were trained to discriminate LSD (0.1 mg/kg, 15-min pretreatment, intraperitoneal injection) from saline as described previously (Fiorella et al. 1995b). An FR10 reinforcement schedule was employed. Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, 83% or more of all responses prior to the delivery of the first reinforcer were on the appropriate lever.

After stimulus control with the training drug was well established, tests were conducted once per week in each animal so long as performance during the remainder of the week did not fall below a criterion level of 83% correct responding.

Test procedure

During test sessions, no responses were reinforced and the session was terminated after the emission of ten responses on either lever. The distribution of responses between the two levers was expressed as the percentage of total responses emitted on the drug-appropriate lever. Response rate was calculated for each session by dividing the total number of responses emitted prior to lever selection, that is, prior to the emission of ten responses on either lever, by the elapsed time. Varying doses of antagonists were administered at a pre-treatment time of 75 min. Antagonists were administered alone, in combination with the training stimulus, LSD (0.1 mg/kg, 15-min pretreatment time), or in combination with (−)DOM (0.4 mg/kg, 75-min pretreatment time). Pretreatment times and doses are based on the results of a previous study (Fiorella et al. 1995b).

Binding assays

Receptor binding assays were carried out by a method previously described (Fiorella et al. 1995a). In brief, frontal cortex (5-HT_{2A}) and choroid plexus (5-HT_{2C}) tissues were homogenized (Dounce tissue grinder) in 50 mM TRIS-HCl (pH 7.4) and centrifuged at 40 000 *g* for 15 min at 4°C. The pellets were resuspended in 40 vol TRIS-HCl buffer pre-warmed to 37°C, and the suspension was incubated at 37°C for 10 min to remove endogenous 5-HT (Nelson et al. 1978). The suspensions were then centrifuged at 40 000 *g* for 15 min and resuspended in ice cold TRIS buffer. After resuspension, centrifugation was repeated and the final pellets were resuspended in 50 mM TRIS-HCl (pH 7.4), 4.0 mM $MgCl_2$, 10 μM pargyline and 0.1% ascorbate. Binding assays were carried out for 30 min, at 30°C for [^{3}H]ketanserin and 37°C for [^{3}H]mesulergine, in a final volume of 0.3 ml consisting of 0.25 ml of tissue suspension, 0.025 ml of the appropriate drug solution, and 0.025 ml of radioligand. Concentration ranges of 0.5–8.0 nM and 0.1–5.0 nM for [^{3}H]mesulergine and [^{3}H]ketanserin, respectively, were used for equilibrium saturation experiments, while 4.0 nM [^{3}H]mesulergine and 3.185 nM [^{3}H]ketanserin were used for competition studies. In previous studies, these conditions resulted in equilibrium binding at all radioligand concentrations. Incubations were terminated with a Brandell cell harvester, and filters, which were pre-soaked with 0.1% PEI, were washed twice with ice cold 50 mM TRIS-HCl (pH 7.4). Filters were incubated overnight with 2.5 ml Ecoscint scintillation cocktail (National Diagnostics) and the amount of bound radioactivity was determined by liquid scintillation spectrophotometry (Beckman LS 6800 Series). Specific binding was defined as the difference in the amount of radioactivity bound in the presence and absence of 100 μM cinanserin (for [^{3}H]ketanserin) or 20 μM 5-HT (for [^{3}H]mesulergine). The data were analyzed by non-linear regression using the program EBDA\ligand (Elsevier BIOSOFT).

Drugs

LSD and (−)DOM were obtained from the National Institute of Drug Abuse. LY53857 maleate, promethazine HCl, thioridazine, cyproheptadine, loxapine succinate, spiperone HCl, mesulergine HCl, and ketanserin tartrate, were purchased from Research Biochemicals International (Natick, Mass.) and 5-HT creatinine sulfate complex was purchased from Sigma (St Louis, Mo.). The following drugs were generously provided by the organizations indicated: pizotyline (Sandoz Pharmaceuticals, E. Hanover, N.J.), pirenpirone and risperidone (Janssen Pharmaceuticals, Belgium), metergoline (Farmaceutica Italia, Italy) cinanserin (Squibb, New Brunswick, N.J.). Stock solutions were prepared by dissolving LY53857, pizotyline, metergoline, and spiperone HCl in a minimal volume of ethanol, ketanserin, risperidone and pirenpirone in a minimal volume of 8.5% lactic acid, and promethazine, thioridazine and loxapine in water. Stock solutions were diluted up to the appropriate concentrations with 0.9% saline. All solutions were injected IP in a volume of 1.0 ml/kg body weight.

Analysis of data

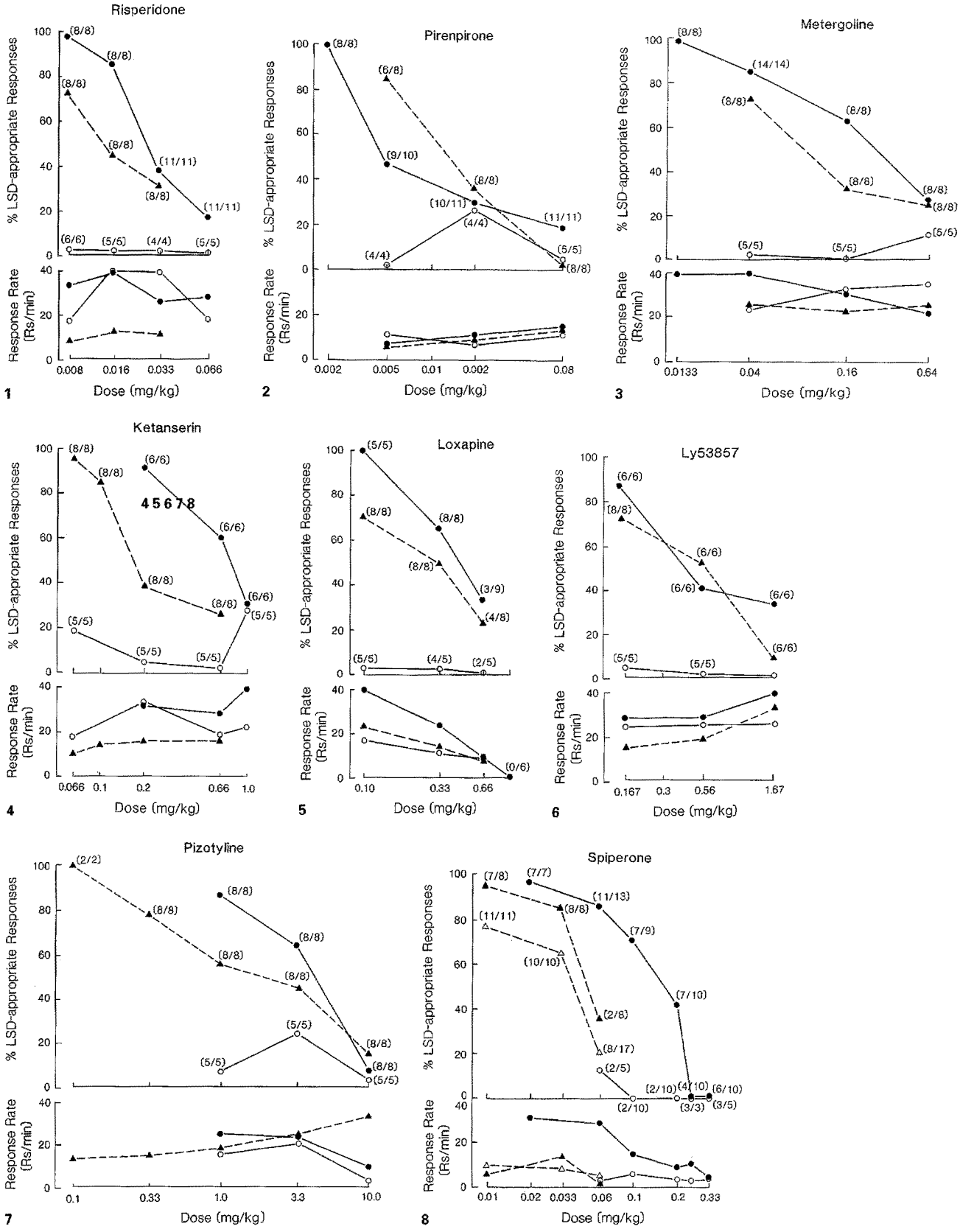
Antagonists were considered in correlation analysis only if (1) The combination of drug and antagonist elicited significantly lower levels of LSD-appropriate responding than the training condition. Significance was determined by individual applications of the paired Wilcoxon's signed ranks test. (2) The antagonist blocked LSD-appropriate responding to levels below 50%. In vivo IC_{50} values were determined by log-logit transformations of the appropriate behavioral data (Rodbard and Frazier 1975). A Pearson product moment correlation analysis was then employed to examine the relationship between in vitro binding affinity at 5-HT_{2A} and 5-HT_{2C} receptors and IC_{50} values for the blockade of LSD-appropriate responding. Regression analysis was performed using the Lotus 1-2-3 spreadsheet program. The significance levels of correlation coefficients were determined using Student's *t*-test for correlations.

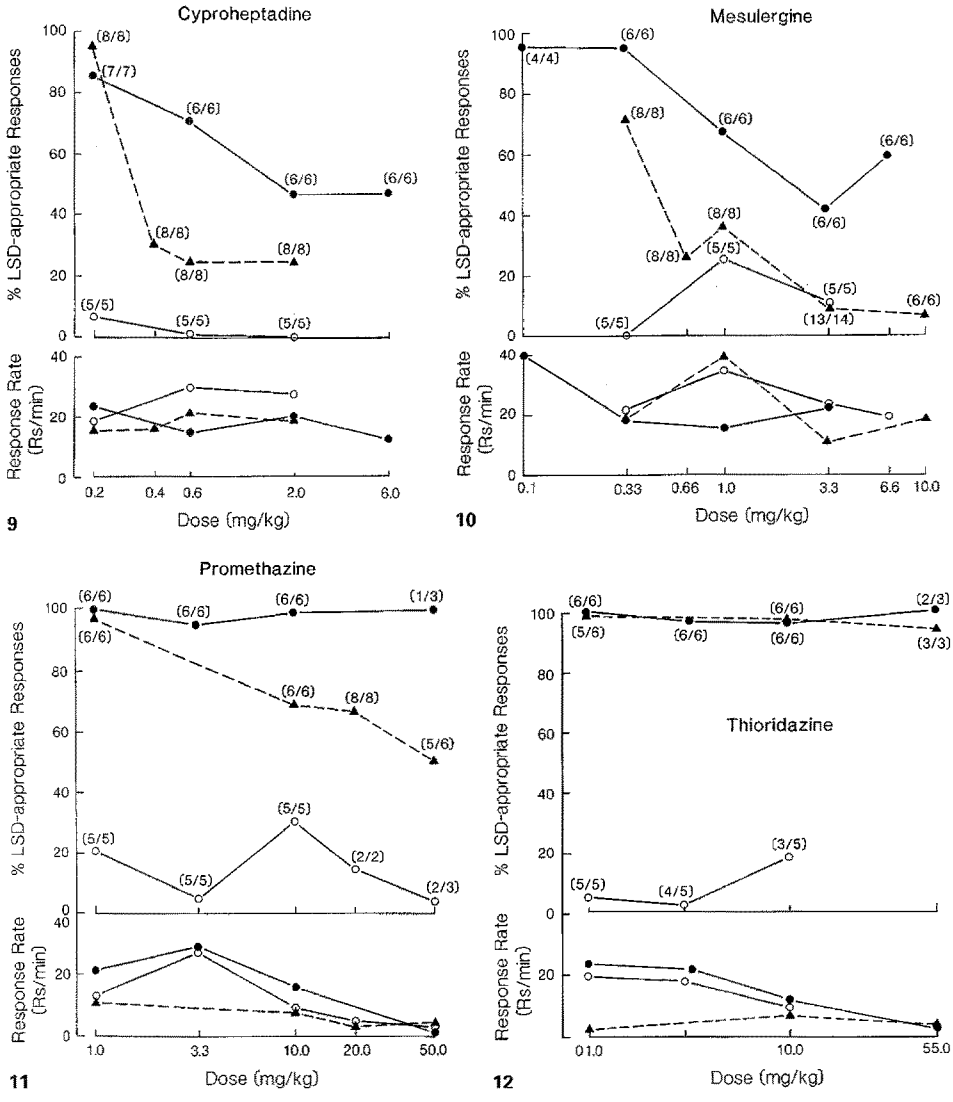
Results

When administered alone, 0.1 mg/kg LSD (15-min pretreatment time) elicited an average of 97% LSD-appropriate responding, while 0.4 mg/kg (−)DOM (75-min pretreatment time) elicited on average 99% LSD-appropriate responding, in the LSD-trained subjects. Administration of vehicle elicited on average 1.4% LSD-appropriate responding.

Risperidone, pirenpirone, metergoline, ketanserin, loxapine, LY53857 and pizotyline produced a dose-dependent antagonism of both the LSD stimulus (0.1 mg/kg, 15-min pretreatment time) and the substitution of (−)DOM (0.4 mg/kg, 75-min pretreatment time) for the LSD stimulus (Figs 1–7) at the doses tested. With the exception of pirenpirone, all of these compounds were more potent antagonists of the LSD-appropriate responding elicited by (−)DOM than LSD (Table 1).

Spiperone produced a dose-dependent inhibition of the LSD stimulus (Fig. 8). However, when spiperone was administered in combination with 0.4 mg/kg (−)DOM, substantial rate suppression resulted in the failure of some subjects to complete test sessions. At the highest dose of spiperone tested (0.06 mg/kg) in combination with (−)DOM (0.4 mg/kg) only two of eight subjects completed the test session. For this reason, the interaction of spiperone with 0.2 mg/kg (−)DOM (75-min pretreatment time) was also studied. The 0.2 mg/kg dose of (−)DOM elicited greater than 90% LSD-appropriate responding when administered alone (Fiorella et al. 1995b). The substitution of 0.2 mg/kg (−)DOM for the LSD stimulus was


Risperidone
Pirenpirone
Metergoline
Ketanserin
Loxapine
Ly53857
Pizotyline
Spiperone
% LSD-appropriate Responses
Response Rate (Rs/min)
Dose (mg/kg)
1
2
3
4
5
6
7
8



Figs 1–12 Dose-inhibition relationships for (*1*) risperidone (*2*) pirenpirone (*3*) metergoline (*4*) ketanserin (*5*) loxapine (*6*) LY53857 (*7*) pizotyline (*8*) spiperone and (*9*) cyproheptadine (*10*) mesulergine (*11*) promethazine (*12*) thioridazine alone (*open circles*), in the presence of LSD (0.1 mg/kg, 15-min pretreatment time) (*closed circles*), in the presence of (−)DOM (0.4 mg/kg, 75-min pretreatment time) (*closed triangles*), or in the presence of (−)DOM (0.2 mg/kg, 75-min pretreatment time) (*open triangles*). The number of subjects completing the test session, and the number of subjects participating in each test session, is expressed as a ratio adjacent to each of the points. *Ordinate*: *upper panel*: mean percentage of responses on the LSD-appropriate lever; *lower panel*: response rate expressed as responses per minute. *Abscissa*: dose of antagonist (mg/kg)

Table 1 Receptor affinity values (K_i's) were determined in vitro from radioligand competition experiments [some data were previously reported (Fiorella et al. 1995a)]. ID_{50} values were determined for the antagonism of the LSD-appropriate responding elicited by LSD (0.1 mg/kg, 15-min pretreatment time) and (−)DOM (0.4 mg/kg, 75-min pretreatment time) by a log-logit transformation of the appropriate in vivo dose-inhibition curves. No antagonism is indicated by "*NA*"

Drugs	K_i [SEM] in nM 5-HT_{2A}	5-HT_{2C}	ID_{50} [mg/kg] vs. LSD	vs. (−)DOM
Risperidone	1.42 (0.24)	18.3 (2.0)	0.032	0.016
Pirenpirone	1.91 (0.40)	58.9 (10.5)	0.004	0.013
Metergoline	2.24 (0.13)	0.57 (0.06)	0.22	0.11
Ketanserin	3.84 (0.60)	118 (24.0)	0.78	0.30
Loxapine	7.85 (1.0)	17.6 (1.6)	0.56	0.41
LY53857	17.5 (2.9)	14.7 (0.45)	0.99	0.60
Pizotyline	10.3 (2.5)	4.18 (1.4)	3.1	1.5
Spiperone	1.75 (0.44)	4700 (1400)	0.19	0.057
Cyproheptadine	4.80 (0.54)	20.6 (5.1)	1.8	0.40
Mesulergine	10.0 (2.7)	3.17 (0.20)	2.3	0.86
Promethazine	124.0 (31.0)	35.9 (3.7)	NA	57.0
Thioridazine	93.6 (18.0)	63.1 (20.0)	NA	NA

completely antagonized by spiperone. When this lower dose of (−)DOM was tested in combination with 0.06 mg/kg spiperone, a greater proportion of subjects completed the test session (8 of 17).

Cyproheptadine produced a partial blockade of both LSD and (−)DOM elicited LSD-appropriate responding (Fig. 9), while mesulergine produced a full blockade of (−)DOM elicited LSD-appropriate responding

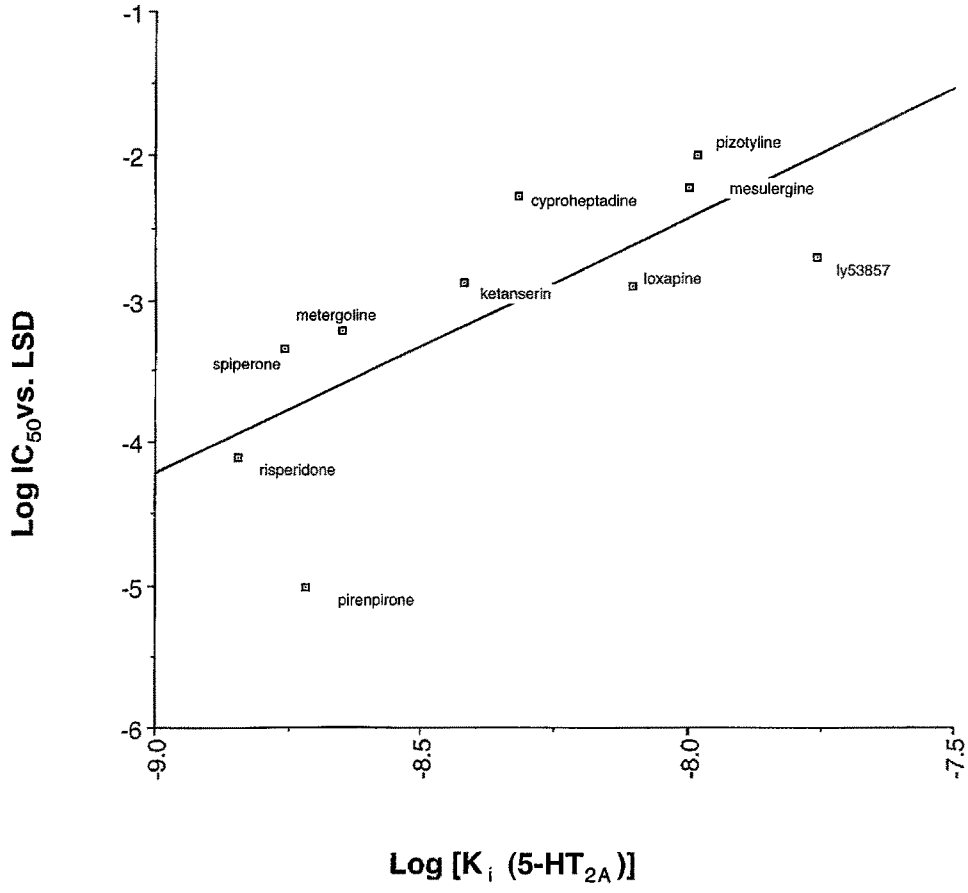


Fig. 13 Correlation between the IC_{50} (mol/kg) for the inhibition of the LSD stimulus and K_i (M) at the 5-HT_{2A} receptor for the series of ten antagonists. Pearson correlation coefficient (r) = +0.75, $P < 0.05$

and a partial blockade of the LSD stimulus (Fig. 10) at the doses tested.

Promethazine and thioridazine both failed to produce any blockade of the LSD stimulus at dosage levels compatible with test session completion (Figs 11 and 12). Promethazine, however, did produce a partial blockade of (−)DOM elicited LSD-appropriate responding. Doses of promethazine which could be administered in combination with (−)DOM were limited by rate suppression and toxicity. Three subjects tested at the highest dose of promethazine (50 mg/kg) died within 24 h of administration.

None of the antagonists studied elicited significant levels of LSD-appropriate responding when administered alone (Figs 1–12).

Dose-inhibition data were subject to log-logit transformation (Rodbard and Frazier 1975). Two in vivo ID_{50} values were determined for each antagonist: one for the blockade of the training stimulus, LSD (0.1 mg/kg, 15-min pretreatment time), and one for the blockade of the LSD-appropriate responding elicited by (−)DOM (0.4 mg/kg, 75-min pretreatment) (Table 1).

Regression analysis of binding and behavioral data

ID_{50} values obtained from combination tests (Figs 1–11, Table 1), were analyzed for correlation with binding data (Table 1), describing the 5-HT_{2A} and 5-HT_{2C} receptor affinities for the series of serotonergic antagonists.

Figure 13 demonstrates the correlation between the K_i values at the 5-HT_{2A} receptor as determined in vitro and the corresponding ID_{50} values for the inhibition of the LSD stimulus as determined in vivo for the series of serotonergic antagonists. 5-HT_{2A} affinity was found to correlate significantly with potency to block the LSD stimulus (r = +0.75, $P < 0.05$).

Figure 14 demonstrates the correlation between the K_i values at the 5-HT_{2A} receptor and the corresponding ID_{50} values for the blockade of (−)DOM elicited LSD-appropriate responding for the series of serotonergic antagonists applied in the correlation analysis of the LSD stimulus. 5-HT_{2A} affinity was found to correlate significantly with potency to block (−)DOM elicited LSD-appropriate responding (r = +0.95, $P < 0.001$). If the IC_{50} value for promethazine is excluded from the correlation analysis, the correlation coefficient becomes 0.88 ($P < 0.01$).

No significant correlation was observed between the K_i values at the 5-HT_{2C} receptor and the corresponding ID_{50} values for either the blockade of the LSD stimulus (r = −0.25) or the blockade of (−)DOM elicited LSD-appropriate responding (r = −0.29) for the series of antagonists.

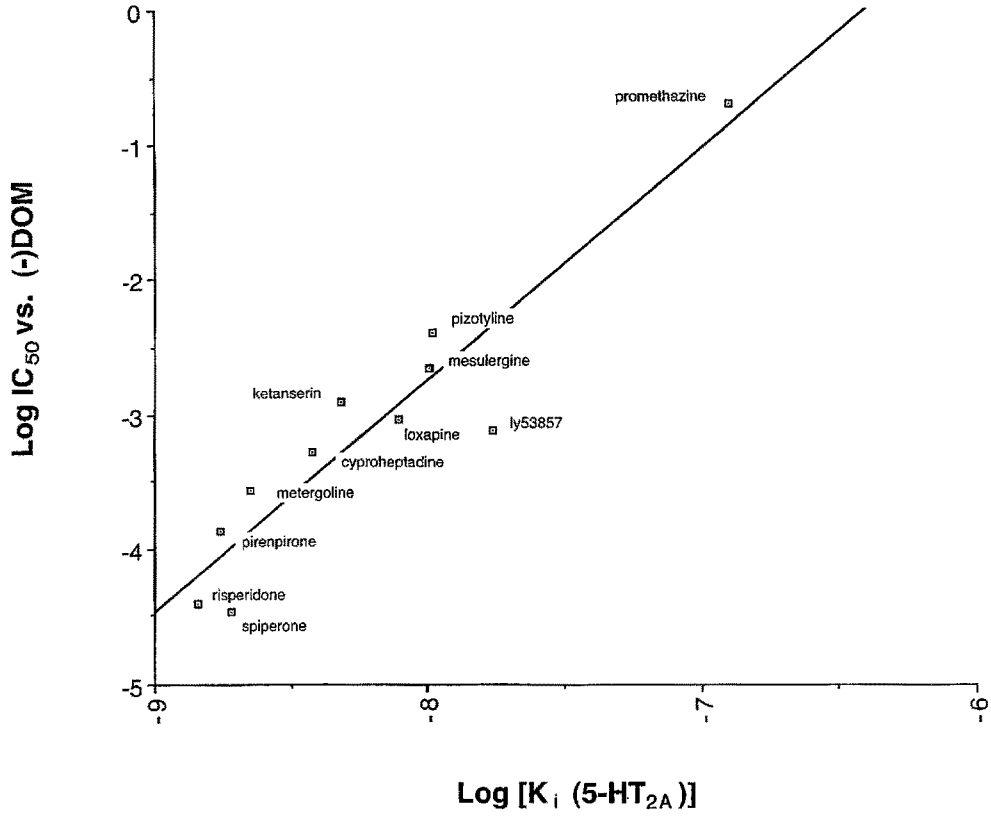


Fig. 14 Correlation between IC_{50} (mol/kg) for the inhibition of the (−)DOM elicited LSD-appropriate responding and K_i (M) at the 5-HT_{2A} receptor for the series of 11 antagonists. Pearson correlation coefficient (r) = +0.95, $P < 0.001$

Discussion

The present study was designed to define the respective roles of the 5-HT_{2A} and 5-HT_{2C} receptors in the stimulus effects of hallucinogens. This was achieved by the application of antagonist correlation analysis to the investigation of the LSD and (−)DOM stimuli in subjects trained to discriminate LSD (0.1 mg/kg, 15-min pretreatment time) from saline.

Consistent with previous reports, pirenpirone (Colpaert and Jannsen 1983; Winter and Rabin 1988), metergoline (Appel and Cunningham 1986), pizotyline (Holohean et al. 1982; Winter and Rabin 1988), risperidone (Meert et al. 1989), ketanserin (Nielsen et al. 1985) and LY53857 (Appel and Cunningham 1986) fully antagonized, cyproheptadine (Colpaert et al. 1982) partially antagonized, and promethazine (Kuhn et al. 1978) did not antagonize the LSD stimulus. In addition, loxapine fully blocked, mesulergine partially blocked and thioridazine did not block the stimulus effects of LSD. We are unaware of other studies which have investigated the interactions of either loxapine, mesulergine, or thioridazine with the LSD stimulus. In contrast to the findings of Cunningham and Appel (1986) spiperone was observed to produce a complete and dose-dependent blockade of the LSD stimulus. The ID_{50} values for the blockade of the LSD stimulus by the series of ten antagonists correlated significantly ($P < 0.05$) with 5-HT_{2A} receptor affinity as determined in vitro (Fig. 13). This positive correlation (r = +0.75) indicates that the in vivo potency of the antagonists to block the LSD stimulus is directly proportional to the in vitro affinity of those same antagonists for 5-HT_{2A} receptors. However, only 56% of the variability in the potency of a given antagonist to block the stimulus effects of LSD can be accounted for by 5-HT_{2A} receptor affinity. This result suggests that while agonist activity at 5-HT_{2A} receptors is of central importance to the stimulus effects of LSD, other receptors may play significant roles. This finding is in accord with biochemical studies which have demonstrated the pharmacology of LSD to be complex and non-selective (see Introduction).

No relationship was observed between the ID_{50} values for the antagonism of the LSD stimulus and 5-HT_{2C} receptor affinity. These results indicate that interactions with 5-HT_{2C} receptors cannot account for the remaining variance in the potencies of the antagonists to block the LSD stimulus. This observation does not, however, exclude the possibility that 5-HT_{2C} receptors play an ancillary role in the stimulus effects of LSD, and possibly an important role in the hallucinogenic effects of LSD in humans.

When studied in humans, the (−)isomer of DOM was reported to elicit LSD-like effects, while the (+)isomer was described to elicit principally non-hallucinogenic subjective effects (Shulgin 1973; Shulgin and Shulgin 1992). LSD and (−)DOM differ structurally, pharmacologically and pharmacodynamically. While the hallucinogenic effects of these two compounds have

been described to be similar, the overall subjective effects elicited by each compound have been reported to differ significantly (Pollard et al. 1960; Jacobsen et al. 1963; Freedman 1968, 1969; Hollister et al. 1969; Shulgin 1973; Kulig 1990; Shulgin and Shulgin 1991). Correspondingly, one would expect that the stimulus properties of these two compounds in the rat might also differ substantially. However, the ability of (−)DOM to substitute for the LSD stimulus suggests that some aspects of the stimulus effects, and thus the relevant in vivo pharmacology of these compounds are shared (Fiorella et al. 1994a). These similarities are of particular interest, as the receptor interactions which mediate the shared aspects of the LSD and (−)DOM stimuli in infra-human species would be expected to correspond with those interactions which mediate their common subjective effects in humans, including their central effect of hallucinosis. Therefore, we propose that substitution of (−)DOM for the LSD stimulus (1) enhances the specificity and relevance of the drug discrimination paradigm to the general mechanism by which indolamine and phenylalkylamine compounds induce hallucinations in humans, and (2) de-emphasizes the idiosyncratic characteristics of the stimulus properties of each compound.

On this basis, antagonist correlation analysis was also applied to investigate the substitution of (−)DOM (0.4 mg/kg, 75-min pretreatment time) for the LSD stimulus. 0.4 mg/kg (−)DOM fully substitutes for the LSD stimulus in a time-dependent manner, eliciting maximal LSD-appropriate responding (99%) at pretreatment times greater than 60 min (Fiorella et al. 1995a). With the exception of promethazine and thioridazine, all antagonists tested fully blocked the substitution of (−)DOM for LSD. Promethazine did produce a partial blockade (48% maximal inhibition) of (−)DOM stimulus, which was sufficient to determine an IC_{50} value. The ID_{50} values for the blockade of (−)DOM elicited LSD-appropriate responding by the series of 11 antagonists correlated significantly ($P < 0.001$) with 5-HT_{2A} receptor affinity as determined in vitro (Fig. 14). This positive correlation ($r = +0.95$) indicates that the in vivo potency of the antagonists to block the (−)DOM elicited LSD-appropriate responding is directly proportional to the in vitro affinity of those same antagonists for 5-HT_{2A} receptors. 89% of the variance in the potency of a given antagonist to block the (−)DOM stimulus in LSD-trained subjects in vivo can be accounted for by the in vitro affinity of that antagonist for 5-HT_{2A} receptors. As previously was the case for the LSD stimulus, no relationship was observed between the ID_{50} values and 5-HT_{2C} receptor affinity. While additional receptor interactions appear to play important roles as mediators of the LSD stimulus, the high degree of correlation between the ID_{50} values for the blockade of (−)DOM elicited LSD-appropriate responding and 5-HT_{2A} receptor affinity indicates that (−)DOM elicited LSD-appropriate responding is mediated predominantly by 5-HT_{2A} agonist activity.

Thus, while traditional studies had failed to differentiate the roles of the 5-HT_{2A} and 5-HT_{2C} receptors in the stimulus effects of hallucinogenic agents, antagonist correlation analysis makes a clear distinction between these two variables. With the exception of spiperone, none of the antagonists in the present study could be termed selective for either receptor, however the series represents a range of 5-HT_2 receptor affinities spanning more than 2 orders of magnitude. Correlating the in vivo ID_{50} values for the series of antagonists with their in vitro affinity values overcomes their individual lack of selectivity. This advantage of correlation analysis is contingent on the lack of a relationship between 5-HT_{2A} and 5-HT_{2C} receptor affinity within the series of antagonists. This criterion was achieved by specifically selecting antagonists which varied structurally. While this parameter can be achieved when antagonists are employed in correlation analysis, often it is not possible with agonists. Agonists acting at a given receptor are generally more structurally constrained than are antagonists. For example, only three major structural classes of compounds have been reported to stimulate 5-HT_2 receptors as measured by the stimulation of PI turnover: phenylalkylamines, indolamines and phenylpiperazines (Conn and Sanders-Bush 1987). As a result, when a series of agonists is chosen for correlation analysis, they are generally very similar both structurally and pharmacologically. This was certainly the case in the agonist correlation analysis performed by Titeler and associates (1988). With the exception of LSD, the agonists chosen for this study were all phenylalkylamines which varied only slightly in structure. As a result, within the series of agonists 5-HT_{2A} and 5-HT_{2C} affinities correlated very strongly ($r = +0.83$) (Glennon et al. 1992), making any differentiation between the relative roles of these two receptors by this method impossible. In addition, correlations employing agonists rather than antagonists may be confounded by the additional pharmacological variable of efficacy.

The present data lend further support to the hypothesis of Glennon and his colleagues that agonist activity at 5-HT_2 receptors is an essential component of the stimulus effects of indolamine and phenylalkylamine hallucinogens (Glennon et al. 1983; Glennon 1990). In addition, the present data extend and refine our knowledge of the role of serotonin in the stimulus effects of hallucinogens by indicating a predominant role for the 5-HT_{2A} receptor subtype.

While the present manuscript was under revision, a report appeared in the literature (Schreiber et al. 1994) which described the role of 5-HT_{2A} agonist interactions in the stimulus effects of the phenylalkylamine hallucinogen (2,5-dimethoxy-4-iodophenyl)2-aminopropane (DOI). In agreement with the present study, these authors conclude that the stimulus effects of DOI

are mediated by agonist interactions with 5-HT_{2A} receptors. These authors report that the correlation between 5-HT_{2A} receptor affinity in vitro and potency to block the DOI stimulus in vivo for a series of eight serotonergic antagonists fails to reach statistical significance ($r = 0.52$, $P > 0.05$). The lack of a significant correlation might be attributed to several factors including the application of quantal dose-response data, the relatively small number of subjects employed to define dose-response relationships, and the application of different antagonists in correlation analysis. In addition, these authors report that a 5-HT_{2A} selective antagonist, MDL 100,907, but not a 5-HT_{2C} selective antagonist SB 200,646A, blocks the DOI stimulus.

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