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Effects of Atypical Antipsychotic Agents on Social Behavior in Rodents

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CORBETT, R., H. HARTMAN, L. L. KERMAN, A. T. WOODS, J. T. STRUPCZEWSKI, G. C. HELSLEY, P. C. CONWAY AND R. W. DUNN. *Effects of atypical antipsychotic agents on social behavior in rodents.* PHARMACOL BIOCHEM BEHAV **45**(1) 9–17, 1993.—There are numerous preclinical screening procedures that are predictive of clinical efficacy for the positive symptoms of schizophrenia but no assays for the negative symptoms such as social withdrawal. In the social interaction test in rats, the atypical antipsychotic drug clozapine (10.0 mg/kg) and two putative atypical agents risperidone (0.0625 mg/kg) and HP 873 (0.5 and 1.0 mg/kg) significantly increased social interaction behaviors between pairs of unfamiliar but not familiar rats. The benzodiazepine diazepam (1.25–5.0 mg/kg) increased social behaviors in both paradigms. Haloperidol, chlorpromazine, raclopride, and SCH23390 decreased social behaviors in these assays. In vitro receptor binding studies revealed that only clozapine, risperidone, and HP 873 displayed dopamine to serotonin affinity ratios for both D_2/5-hydroxytryptamine$_2$(5-HT_2)/ and D_1/5-HT_{1A} of greater than or equal to 12.9 and 1.0, respectively. The present study suggests that antipsychotic agents that may be effective in social withdrawal can be identified in this modified social interaction paradigm. Further, our data suggests that a compound's effectiveness for the treatment of social withdrawal is at least in part due to its relative affinity for binding to dopamine D_1 and serotonin 5-HT_{1A} receptors.

Social behavior Antipsychotic Negative symptoms Serotonin Dopamine

THERE are two syndromes of schizophrenia: one characterized by the positive symptoms of delusions, hallucinations, and bizarre or disorganized behavior; the second characterized by the negative symptoms such as flattening of affect, poverty of speech, impaired attention, and social withdrawal (1, 11,12). Although there are preclinical screening assays that are predictive for an antipsychotic agent's potential efficacy for the positive symptoms of schizophrenia (24), procedures for prediction of efficacious compounds for negative symptoms are lacking. Clozapine and risperidone have been characterized as atypical antipsychotic agents based upon their reduced extrapyramidal side effect (EPS) liability in patients (27,35). This low EPS liability is correlated in vivo to a lack of cataleptic behavior in rodents and in vitro to a higher affinity for serotonin [5-hydroxytryptamine$_2$ (5-HT_2)] relative to dopamine D_2 receptors (5,26,30,34). In addition to these properties, clozapine has been shown to be effective in treatment-resistant schizophrenics with significant improvements observed in both positive and negative symptomatology (15, 27,32,33,39). Early clinical studies have reported that the combined serotonin 5-HT_2 and dopamine D_2 receptor antagonist risperidone has shown antipsychotic efficacy and improvement of some negative symptoms (3,8). A third compound, HP 873, has been shown by preclinical measures to possess atypical properties and potential effectiveness for negative symptoms (42,45).

The present investigation proposes the utility of a modified social interaction test for the preclinical prediction of "atypical/negative symptom" antipsychotic agents, such as clozapine, risperidone, and HP 873, that may be effective in treating social withdrawal. The social interaction test was initially developed as a model for the prediction of potential anxiolytic agents (19). We modified this procedure to differentiate between the activity of atypical antipsychotic agents with potential clinical efficacy for negative symptoms from the classic antipsychotic agents haloperidol and chlorpromazine and from the selective D_1 and D_2 antagonists SCH23390 and raclopride, respectively. In addition, the effects of clozapine, risperidone, and HP 873 in this model were further differentiated from the anxiolytic effects of the benzodiazepine diazepam.

METHOD

Subjects

Male Wistar rats and male CD-1 mice (Charles River Laboratories, Wilmington, MA) were housed under standard laboratory conditions as outlined in the NIH Guide for the Care

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and Use of Laboratory Animals (National Institutes of Health Publication 85-23, Revised 1985) with a 12 L : 12 D cycle and allowed free access to water.

Drugs

Chlorpromazine (Smith Kline & French, Philadelphia, PA), clozapine (Sandoz, Inc., East Hanover, NJ), diazepam (Hoffmann-LaRoche, Nutley, NJ), haloperidol (McNeill Pharmaceuticals, Fort Washington, PA), HP 873 (Hoechst-Roussel, Somerville, NJ), raclopride (Astra, Worcester, MA), ritanserin (Janssen), risperidone (Janssen) 8-hydroxy-2-(di-*n*-propylamino) tetralin (8-OH-DPAT), and SCH23390 (Research Biochemicals Inc., Natick, MA) were administered in these studies. Compounds were either dissolved or suspended in distilled water with a drop of Tween-80. The final volume was prepared to account for salt content and the dosage was expressed as 100% base. Mice were administered compounds in a dosage volume of 1 ml/100 g while rats received compound in a dosage volume of 1 ml/kg.

Assays

Apomorphine-Induced Climbing Mouse Assay (CMA). Male CD-1 mice (20–30 g) were assigned to groups of 8 and placed individually in wire mesh stick cages (4 × 4 × 10 in.) (10). After 1-h habituation to the cage, animals were IP administered compounds 30 min prior to testing (except haloperidol: 60 min pretreatment). Apomorphine was then administered SC at 1.5 mg/kg, a dose that causes a stereotyped climbing behavior in all mice. For evaluation of climbing behavior, readings were taken at 10, 20, and 30 min after apomorphine administration, according to the following scale: mice with four paws on bottom = 0; mice with one to two paws on vertical wall = 1; mice with three to four paws on vertical wall = 2. The climbing scores were individually totaled (maximum score = 6 per animal), and the mean score of each group was compared to the mean score of the control group, which was set to equal 100%. ED_{50} values and 95% confidence limits were then calculated (31).

Catalepsy (CAT). Groups of 10 male Wistar rats were used in this procedure. Catalepsy was scored as previously described (9). Drugs were administered IP with a 60-min pretreatment time (except SCH23390: SC administration, 30 min pretreat). The test consisted of placing an individual rat in a white translucent plastic box (26 × 20 × 15 cm) with a wooden dowel mounted horizontally 10 cm from the floor and 4 cm from one end of the box. The floor was covered with approximately 1 cm of bedding material. At the end of a 1-min acclimation period, each rat was gently grasped around the shoulders and under the forepaws and carefully placed on the bar. The latency (in seconds) for the rat to remove both paws from the bar was recorded for a maximum of 180 s. For every 20 s that the animal maintained the cataleptic posture, it received one point such that maximum catalepsy was represented by a total of nine points. ED_{50} and 95% confidence limits were calculated (31).

Social Interaction Test (SI). For the SI test (19), naive male Wistar rats (250–300 g) were housed in pairs for 10 days prior to the start of the test. The SI test consisted of familiarizing each pair (cagemates) of rats to the arena (50 × 50 × 30 cm) for a period of 8 min on 2 consecutive days. In the unfamiliar rat paradigm, on day 3 each rat was randomly assigned, according to weight, to an unfamiliar partner in groups of 12 animals (six pairs) that were subsequently IP administered the appropriate drug 30 min prior to testing. These rats were then returned to their home cage with their original cagemate until testing. In the familiar rat paradigm, cagemates were IP administered compounds (30 min pretreatment) and returned to their home cage.

For each paradigm, either unfamiliar or familiar pairs of rats were placed in the test arena and observed for SI behavior and overall motor activity for 5 min with a summed score totaled for each parameter per pair of rats. SI time (seconds) per pair of rats was measured as time spent sniffing partner, climbing over and crawling under partner, mutual grooming, genital investigation, and following and walking around partner. Aggressive behavior (biting, boxing, and pulling each other) was not considered SI behavior. Also passive social contact was not counted as SI, that is, if animals were next to each other for more than 10 s and did not actively interact the scoring was discontinued until movement resumed. Motor activity was measured by counting the number of rears (lifting of both front paws) and walks (of one body length) per pair of rats. Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test.

Biochemistry

Radiolabeled ligand binding studies for dopamine D_1 and D_2 and serotonin 5-HT_{1A} and 5-HT_2 receptors were performed according to the following established methods. Male Wistar rats (125–225 g) were sacrificed and appropriate areas of the brains rapidly removed at 4°C. For each binding assay, appropriate brain tissue was chosen based upon ^3H ligand selectivity and specificity based upon receptor densities and pharmacological activity of these sites. Each tissue sample was weighed and homogenized by a Polytron (PT-10) at a setting of 5 for 15 s in a Tris buffer (0.05 M, pH 7.7). The homogenate was then centrifuged at 48,000 × *g* for 15 min in a Sorvall RC5B equipped with an SS-34 rotor. The pellet was resuspended, homogenized, and centrifuged as above. The tissue for the 5-HT_{1A} assay was first incubated for 10 min at 37°C to remove any endogenous serotonin and then recentrifuged as above. The final pellets were then resuspended in incubation buffer and appropriate volumes. Radiolabeled ligand studies for dopamine D_1 and D_2 and serotonin 5-HT_{1A} and 5-HT_2 receptors were performed according to the following standard methods that accounted for their respective incubation time and temperature conditions (Table 1): dopamine D_1 receptor (23); dopamine D_2 receptor (30); serotonin 5-HT_{1A} receptor (36); serotonin 5-HT_2 receptor (37). The incubations were stopped by rapid filtration through a Whatman GF/B (Whatman, Clifton, NJ) in a Brandel M-24 cell harvester and washed three times with 5 ml ice-cold 0.05 M Tris buffer. The filters were dried overnight and then counted by adding 10 ml Liquiscent scintillation cocktail and placed in a Beckman LS-3801 scintillation counter (Beckman Instruments, Fullerton, CA) and radioactivity was counted for 2 min. The percentage specific binding was calculated as the difference of total binding vs. binding in the presence and absence of appropriate specific displacer. The IC_{50} values for each compound were calculated from the percent specific binding at each drug concentration using linear regression (31). The IC_{50} values were then transformed into K_i values (6).

RESULTS

Table 2 summarizes the effects of the antipsychotic agents tested, as well as diazepam, ritanserin, and 8-OH-DPAT, in the CMA and CAT assays. A ratio of the ED_{50} values in CAT vs. CMA activity was generated for each agent tested.

TABLE 1

RECEPTOR BINDING METHODOLOGY

Receptor	Ligand [nM]	Tissue (μg protein/ml)	Specific Displacer	Temperature (°C)	Incubation Time (min)
D_1	[^{3}H]-SCH23390 (0.5)	Striatum (250)	*d*-Butaclamol (1 μM)	37°	30
D_2	[^{3}H]-Spiroperidol (0.14)	Striatum (250)	*d*-Butaclamol (2 μM)	37°	20
5-HT_{1A}	[^{3}H]-DPAT (0.5)	Hippocampus (200)	Serotonin (10 μM)	25°	10
5-HT2	[^{3}H]-Spiroperidol (1.5)	Frontal cortex (250)	Methysergide (5 μM)	37°	10

Haloperidol, chlorpromazine, SCH23390, and raclopride had a CMA/CAT ratio of less than 5, while risperidone and HP 873 had ratios of 95.0 and 323.1, respectively. A ratio for clozapine could not be determined as the compound was devoid of cataleptic activity at doses up to 80 mg/kg, while higher doses were lethal. Diazepam, ritanserin, and 8-OH-DPAT were inactive in both tests.

Figure 1 summarizes the effects of haloperidol at 0.05–0.25 mg/kg (A), chlorpromazine at 1.25–5.0 mg/kg (B), SCH23390 at 0.0325–0.125 mg/kg (C), and raclopride at 0.315–1.25 mg/kg (D) on social interaction behavior and overall motor activity in the unfamiliar and familiar rat paradigms. At the doses tested, these agents only decreased SI behavior in both unfamiliar and familiar rats and in overall motor activity. Significant sedation limited testing to the highest doses shown.

The effects of clozapine, risperidone, HP 873, and diazepam on social interaction behavior in unfamiliar and familiar rats are shown in Fig. 2. Clozapine at 10 mg/kg significantly increased SI behavior by 39% in unfamiliar rats while being ineffective in familiar rats (Fig. 2A). At 20 mg/kg (data not

TABLE 2

EFFECTS OF ANTIPSYCHOTIC AGENTS, DIAZEPAM, RITANSERIN AND 8-OH-DPAT IN THE CMA AND CAT TEST

	ED_{50} and 95% Confidence Limits		
Compound	Climbing Behavior (mg/kg)	Catalepsy (mg/kg)	Ratio (CAT/CMA)
Haloperidol	0.13 (0.11–0.15)	05 (0.44–0.55)	3.8
Chlorpromazine	1.32 (1.2–1.5)	2.7 (0.27–27.0)	2.1
SCH23390	0.16 (0.14–0.2)	0.05 (0.02–1.1)	0.3
Raclopride	0.6 (0.52–0.70)	2.8 (1.0–8.3)	4.7
Clozapine	12.3 (10.8–14.5)	0% at 80mg/kg*	ND
Risperidone	0.06 (0.05–0.08)	5.7 (3.7–8.6)	95.0
HP 873	0.095 (0.09–0.10)	30.7 (19.6–48.1)	323.1
Diazepam	Not active up to 40 mg/kg	Not active up to 40 mg/kg	ND
Ritanserin	Not active up to 20 mg/kg	Not active up to 40 mg/kg	ND
8-OH-DPAT	Not active up to 5.0 mg/kg	Not active up to 10 mg/kg	ND

Mice were administered compounds (mg/kg, IP) 30 min prior to testing (except haloperidol: 60 min pretreatment) for CMA and rats were administered compounds (mg/kg, IP) 60 min prior to testing for catalepsy. ED_{50} values and 95% confidence limits were calculated by means of the Litchfield and Wilcoxon (31) method. n = 8 mice per group for CMA and 10 rats per group for catalepsy.

ND, not determined.

*Highest dose tested; toxicity and lethality were observed at higher doses.

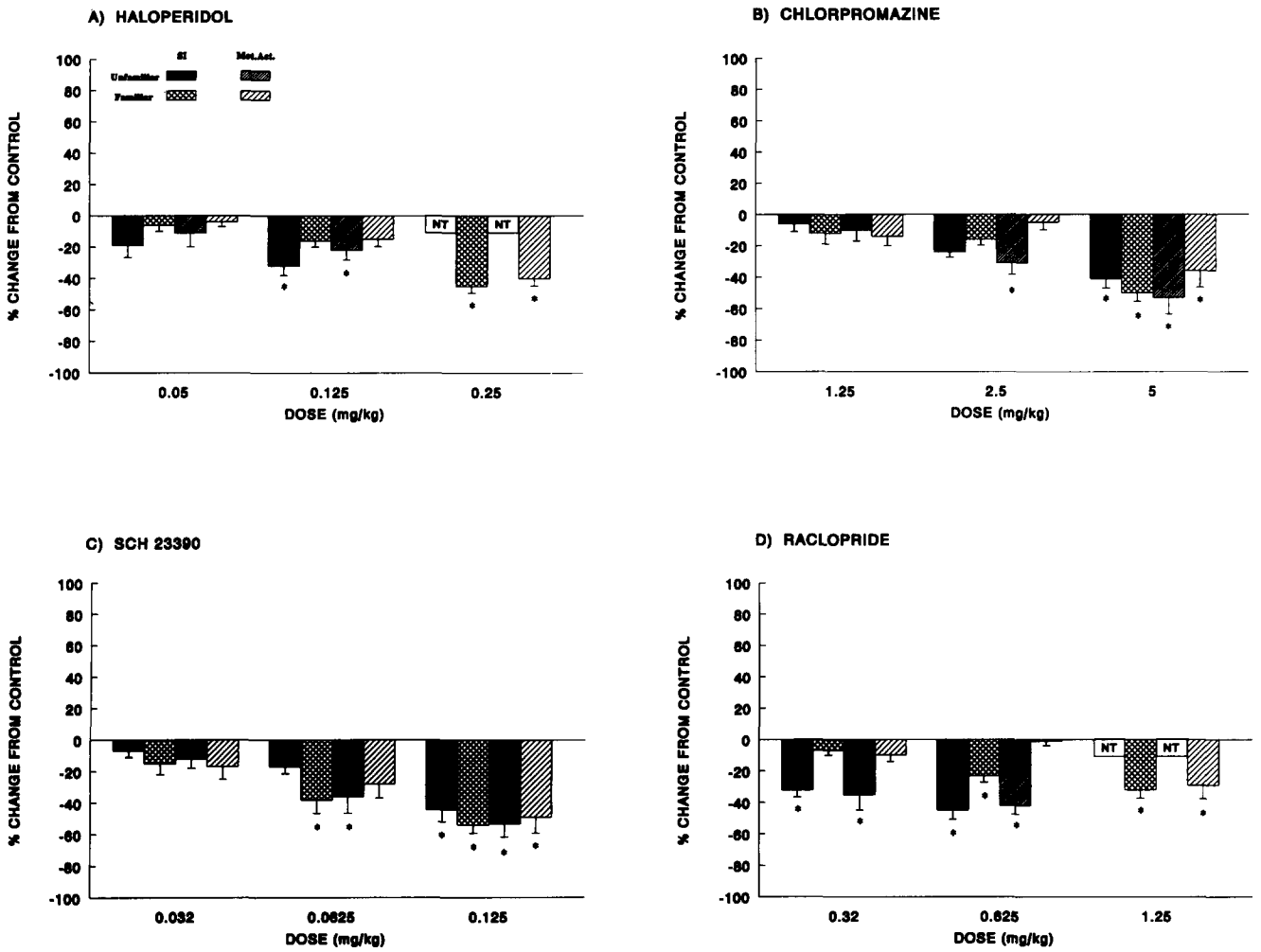

FIG. 1. Comparison of the effects of haloperidol, chlorpromazine, SCH23390, and raclopride in the unfamiliar and familiar social interaction (SI) paradigms as measured by mean percentage change from control (± SEM) Rats were administered (mg/kg, IP) compounds 30 min prior to testing n = six pairs per group. *$p < 005$, one-way ANOVA followed by Dunnett's test NT, not tested.

shown), significant sedation limited SI activity. Risperidone at 0.0625 mg/kg (Fig. 2B) significantly increased SI behavior in unfamiliar rats (+25%) but was ineffective in familiar rats at the doses tested. Significant sedation was observed at higher doses. HP 873 at both 0.5 and 1.0 mg/kg (Fig. 2C) significantly increased SI behavior in unfamiliar rats by 25 and 30%, respectively. There was no effect on social interaction in familiar rats. Significant sedation was noted at the 2.5-mg/kg dose. On the other hand, diazepam at 1.25 and 2.5 mg/kg (Fig. 2D) significantly increased SI behavior in unfamiliar rats by 25 and 64%, respectively, and at 5.0 mg/kg significantly increased SI by 28% in familiar rats. Significant sedation was observed at 2.5 mg/kg for unfamiliar rats and at 5.0 mg/kg for familiar rats.

Ritanserin failed to increase social behaviors in either the unfamiliar or familiar rat paradigm, while significant sedation was observed at higher doses (Fig. 3). However, the 5-HT_{1A} agonist 8-OH-DPAT (0.125–0.25 mg/kg) significantly increased social behaviors by 106 and 114%, respectively, in the unfamiliar rat paradigm, while having no effect in the familiar rat paradigm.

Table 3 summarizes the results of the radiolabeled ligand binding studies for the compounds tested at the D_1, D_2, 5-HT_{1A}, and 5-HT_2 receptor sites. As a measure of antipsychotic drug atypicality, the D_2/5-HT_2 receptor binding ratios of clozapine, risperidone, and HP 873 were determined to be greater than 12. The selective D_1 receptor antagonist SCH23390 displayed a weak affinity for the D_2 receptor site ($K_i > 2.0$ μM) and as a consequence its D_2/5-HT_2 ratio was found to be >18. Haloperidol and chlorpromazine had D_2/5-HT_2 receptor binding ratios of less than 3. The D_2 antagonist raclopride displayed weak affinity for the 5-HT_2 receptor binding site (>2.0 μM) such that the D_2/5-HT_2 was not determined. Diazepam exhibited weak receptor binding affinity for all receptor binding sites studied in this investigation. The 5-HT_2 antagonist ritanserin only had potent affinity for the 5-HT_2 receptor site and weak affinity for the D_2 receptor and as a consequence a D_2/5-HT_2 ratio of 640.

The relative affinity of these compounds for the D_1 and 5-HT_{1A} receptor binding sites were also compared. Clozapine, risperidone, and HP 873 exhibited D_1/5-HT_{1A} receptor binding ratios of 1.1, 1.0, and 3.25, respectively. On the other

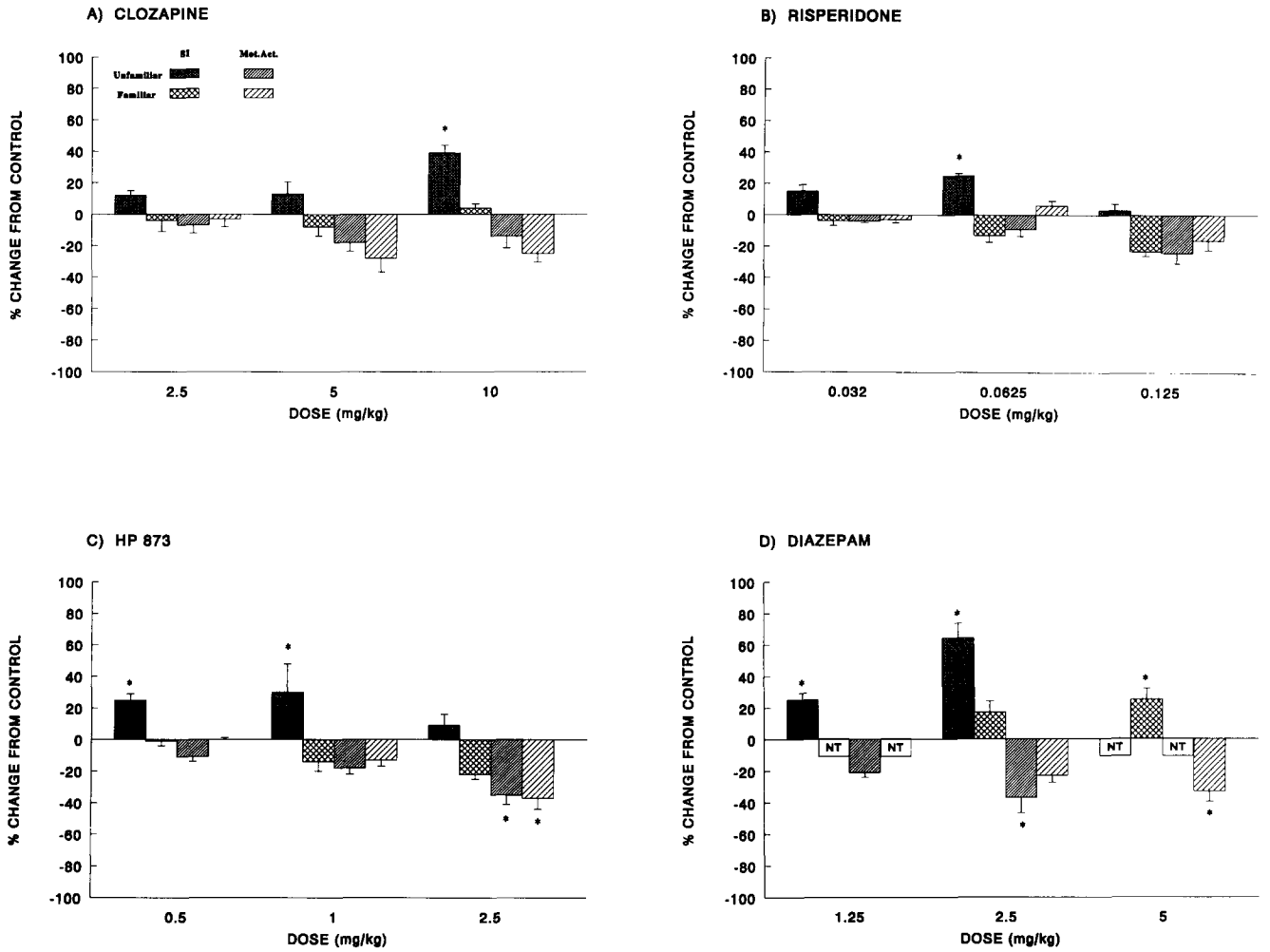

FIG. 2. Comparison of the effects of clozapine, risperidone, HP 873, and diazepam in the unfamiliar and familiar social interaction (SI) paradigms as measured by mean percentage change from control (±SEM) Rats were administered (mg/kg, IP) compounds 30 min prior to testing n = six pairs per group $*p < 005$, one-way ANOVA followed by Dunnett's test NT, not tested.

hand, haloperidol, chlorpromazine, and SCH23390 produced ratios of <0.25, < 0.1, and 0.001, respectively. Because of the weak affinity of raclopride, diazepam, and ritanserin for the D_1 and 5-HT_{1A} receptors, a D_1/5-HT_{1A} receptor binding ratio was not determined. The 5-HT_{1A} agonist 8-OH-DPAT displayed selective affinity for the 5-HT_{1A} receptor site and as a consequence had a D_1/5-HT_{1A} ratio of greater than 1,000.

DISCUSSION

One of the negative symptoms of schizophrenia involves psychosocial deficits that inhibit a schizophrenic patient's ability to interact in normal everyday social circumstances and settings and may impede therapeutic recovery and subsequent reassimilation into society. Therefore, for optimal effectiveness an antipsychotic agent should affect both the positive symptoms of schizophrenia as well as some of the negative symptoms, such as social withdrawal. One setback in the development of antipsychotic agents has been the lack of reliable procedures to predict a compound's effectiveness for treating negative symptoms. To address this issue, the present study presents a modification of the classic SI paradigm in rats. We now report that the atypical antipsychotic agents clozapine and risperidone, which have been reported to be more effective than conventional antipsychotic agents for negative symptomatology, significantly increased social interaction behavior in unfamiliar but not familiar rats. Further, the putative atypical antipsychotic HP 873 also increased social behavior in rats in this manner. All these agents increased social behavior at doses similar to those that produced efficacy in preclinical antipsychotic screening procedures. In contrast, the typical antipsychotic agents haloperidol and chlorpromazine and the selective D_1 and D_2 antagonists, SCH23390 and raclopride, respectively, decreased social behavior in unfamiliar rats. In the familiar rat paradigm, clozapine, risperidone, and HP 873 had no effect on social behavior, while the typical agents decreased this behavior. Therefore, in the unfamiliar rat paradigm, where the novel rat serves as an anxiogenic or stressful stimulus, only clozapine, risperidone, and HP 873 effectively increased social behavior. The increase of this behavior by

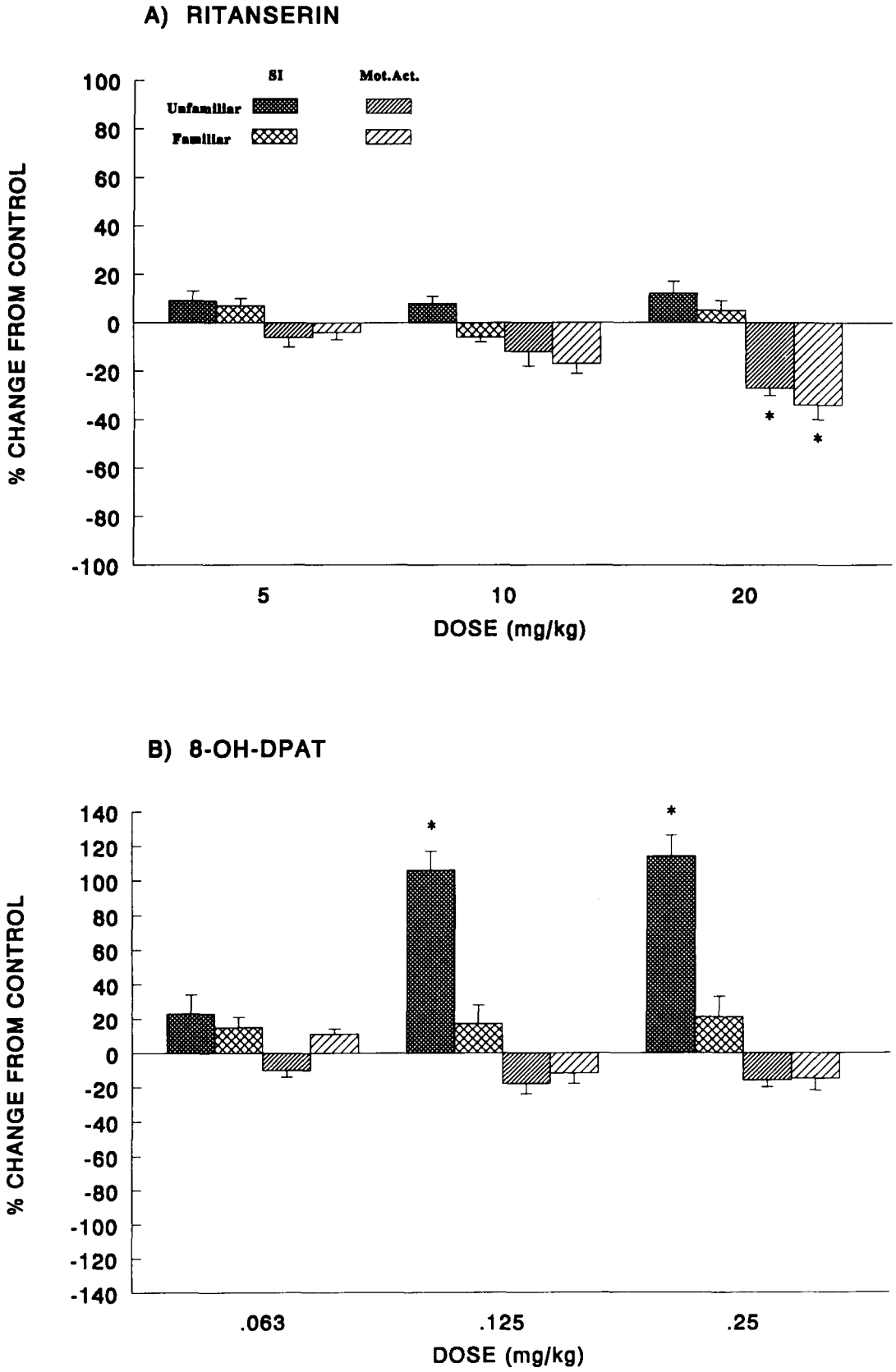

FIG. 3. Comparison of the effects of ritanserin and 8-OH-DPAT in the unfamiliar and familiar social interaction (SI) paradigms as measured by mean percentage change from control (± SEM) Rats were administered (mg/kg) ritanserin 30 min prior and 8-OH-DPAT 15 min prior to testing n = six pairs per group $^*p < 005$, one-way ANOVA followed by Dunnett's test.

TABLE 3

COMPARISON OF THE K_i (μM) VALUES FOR RECEPTOR BINDING TO DOPAMINE D_1 AND D_2 RECEPTOR BINDING SITES AND SEROTONIN 5-HT_{1A} AND 5-HT_2 RECEPTOR BINDING SITES

	K_i (μM) values				Ratios	
Compound	D_1	D_2	$5HT_{1A}$	$5HT_2$	$D_2/5HT_2$	$D_1/5HT_{1A}$
Haloperidol	0.52 ± 0.1	0.013 ± 0.002	>2.0	0.045 ± 0.009	0.29	<0.25
Chlorpromazine	0.26 ± 0.06	0.033 ± 0.006	>2.0	0.012 ± 0.004	2.75	<0.1
SCH 23390	0.0003 ± 0.00001	>2.0	0.31 ± 0.05	0.056 ± 0.015	>18	0.001
Raclopride	>2.0	0.06 ± 0.01	>2.0	>2.0	ND	ND
Clozapine	0.69 ± 0.08	0.79 ± 0.1	0.64 ± 0.1	0.061 ± 0.04	12.9	1.1
Risperidone	0.55 ± 0.07	0.02 ± 0.001	0.57 ± 0.1	0.0014 ± 0.0009	14.3	1.0
HP-873	0.546 ± 0.08	0.054 ± 0.008	0.168 ± 0.02	0.0031 ± 0.002	17.4	3.25
Diazepam	>2.0	>2.0	>2.0	>2.0	ND	ND
Ritanserin	>2.0	0.64 ± 0.23	>2.0	0.001 ± 0.0004	640	ND
8-OH-DPAT	>2.0	>2.0	0.002 ± 0.0002	>2.0	ND	>1000

Relative ratio values of K_i for D_2 / 5-HT_2 and D_1 / 5-HT_{1A} were generated for each compound. n = mean ± SE of at least three experiments. ND, not determined.

these atypical agents was differentiated from the anxiolytic effects of diazepam that increased social behavior in both the unfamiliar and familiar rat paradigms.

In the clinical setting, all antipsychotic agents affect both positive and negative symptoms to some degree (28). However, clozapine has been reported to positively affect resocialization more effectively than other antipsychotic agents (15,27). Risperidone's reported effectiveness on some negative symptoms may be related to its 5-HT_2 antagonism and consequent effects on anergia (3,8). Although clozapine, risperidone, and HP 873 exhibited a unique ability amongst the antipsychotic agents tested to increase social behavior in unfamiliar rats, the mechanism of action responsible for these effects remains uncertain.

Receptor binding studies have shown that while clozapine has relatively weak but equal affinity for both the dopamine D_1 and D_2 receptor sites risperidone and HP 873 have greater affinity for the D_2 site. The typical antipsychotic agents tested in this study have dopamine receptor binding profiles similar to risperidone and HP 873, suggesting that the relative affinity for D_1 vs. D_2 sites is not related to the observed increases in social interaction behaviors. Also, the selective D_1 and D_2 receptor antagonists SCH23390 and raclopride, respectively, only decreased social interaction behavior. All these compounds also have affinity for the serotonin 5-HT_2 receptor site. However, because the selective 5-HT_2 receptor antagonist ritanserin had no effect on social behavior 5-HT_2 antagonism alone does not enhance social behavior in rats.

Antipsychotic atypicality defined as reduced EPS liability has been predicted based upon a higher affinity for the receptor binding to serotonin 5-HT_2 receptors relative to dopamine D_2 receptors (26,34) as well as being relatively devoid of cataleptic behavior in rodents (22). Clozapine, risperidone, and HP 873 all possess this favorable ratio (D_2/5-HT_2 > 12) and preclinically are devoid of cataleptic effects at doses that are effective on social behaviors (26,35,42).

Clinically, clozapine and risperidone have exhibited reduced EPS liability (8,26,35). Typical antipsychotic agents do not possess a favorable D_2/5-HT_2 ratio, produce catalepsy in rodents, and clinically possess EPS liability. Even though SCH23390 exhibits a favorable D_2/5-HT_2 ratio, it has been suggested previously that the 5-HT_2 antagonist properties of this compound would have minimal effects at doses that achieve D_1 receptor blockade (4). In fact, SCH23390 produced catalepsy in rats at doses similar to its CMA activity. As we previously suggested, 5-HT_2 antagonism alone is probably not sufficient to induce social behavior and, likewise, a favorable D_2/5-HT_2 ratio does not appear to be solely responsible for the observed increases in social behavior because SCH23390 does not increase social behavior.

It has been previously shown that 5-HT_{1A} agonists increased social behaviors in unfamiliar rats (16). The present results show that both the atypical as well as the typical antipsychotic agents possess various receptor binding affinities for the 5-HT_{1A} receptor site. Because typical antipsychotic agents do not increase social behaviors but possess 5-HT_{1A} binding properties, 5-HT_{1A} affinity per se in an antipsychotic agent may not be sufficient to affect social behavior. Therefore, the question remains; Why do only the "atypical/negative symptom" antipsychotic agents positively affect social behaviors while the typical antipsychotic agents are devoid of this property?

Social behavior has been attributed to higher-level functioning of the frontal cortex (18). Moreover, it has been shown that 5-HT_{1A} receptor sites and a predominance of dopamine D_1 compared to D_2 receptor sites are present in the prefrontal cortex (13,17,20,21,38). In addition, it has recently been shown that there was a significant increase in the number of 5-HT_{1A} receptor sites in the prefrontal and temporal cortices of both unmedicated and medicated schizophrenic patients (21). Although 5-HT_{1A} agonists such as buspirone have been relatively ineffective against the positive symptomatology of schizophrenia (2,25), there have been reports that suggest these agents were effective in reducing stress-induced psychosocial deficits in chronic schizophrenic patients (25,40).

Therefore, the positive effects on social behavior in rats produced by clozapine, risperidone, and HP 873 may be attributed to a D_1/5-HT_{1A} receptor binding ratio of greater than or equal to 1. That is, antipsychotic agents effective for treating the negative symptoms of schizophrenia possess equal or greater 5-HT_{1A} receptor binding affinity relative to that for D_1 receptors. On the other hand, all of the other antipsychotic agents tested in the present study have D_1/5-HT_{1A} ratios of less than 0.1. Compounds that possess more potent 5-HT_{1A}

agonism compared to D_1 antagonism may show positive effects on social behavior by preferentially activating 5-HT_{1A} receptors in the prefrontal cortex compared to blockade of D_1 receptors in this region (43). However, greater D_1 receptor antagonism compared to 5-HT_{1A} agonism in this region may prevent an increase in social behavior. The clinical relevance of this hypothesis may be related to reports of dopaminergic cortical hypofrontality observed in schizophrenic patients displaying negative symptomatology (14). We speculate that compounds such as clozapine, risperidone, and HP 873 may show better efficacy in negative symptoms and, in particular, social withdrawal in part due to a favorable combination of greater 5-HT_{1A} receptor agonism in relation to D_1 receptor antagonism. Other explanations for this phenomenon cannot be excluded. For example, molecular cloning techniques have revealed a D_5 receptor similar to the D_1 receptor in the frontal cortex that may be important for dopaminergic activity (7,41). In addition, clozapine has been shown to bind with highest affinity to the D_4 receptor subtype (44). Future studies may reveal that the unique profile of clozapine and other atypical/negative symptom agents may be due to selective interactions at these sites.

In conclusion, the present study provides evidence to show that by comparing the effects of antipsychotic agents in unfamiliar vs. familiar rats in the social interaction assay a preclinical empirical differentiation can be made between atypical/negative symptom and other antipsychotic agents. Further, this activity may be due to the greater serotonin 5-HT_{1A} receptor binding affinity relative to that for dopamine D_1 receptors. This type of antipsychotic agent may enhance therapeutic efficacy in schizophrenics and aid in the resocialization of a patient into society.

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REFERENCES

1. Andreasen, N. K. Negative vs positive schizophrenia: Definition and reliability. Arch. Gen. Psychiatry 39:744–748; 1982.
2. Basse-Tomusk, A.; Rebec, G. Ipsapirone depresses neuronal activity in the raphe nucleus and the hippocampal formation. Eur. J. Pharmacol. 130:141–149; 1986.
3. Bersani, G.; Bressa, G. M.; Meco, G.; Marini, S.; Pozzi, F. Combined serotonin 5-HT_2 and dopamine D_2 antagonism: Clinical, extrapyramidal and neuroendocrine response in a preliminary study with risperidone (R64766) Hum. Psychopharmacol. 5:225–231; 1990.
4. Bischoff, S.; Heinrick, M.; Krauss, J.; Sills, M. A.; Williams, M.; Varsout, A. Interaction of the D_1 receptor antagonist SCH 23390 with central 5-HT system: Radioligand binding studies measurements of biochemical parameters and effects on L-5HTP syndrome. J. Receptor Res. 8:107–120; 1988.
5. Blelch, A.; Lynn-Brown, S.; Kahn, R.; Van Praag, H. M. The role of serotonin in schizophrenia. Schizophrenia Bull. 14:297–315; 1988.
6. Cheng, Y. C.; Prusoff, W. H. Relationship between the inhibition constant (Ki) and the concentration of inhibitor which causes 50 percent inhibition of an enzymatic reaction. Biochem. Pharmacol. 22:3099–3108; 1973.
7. Civelli, O.; Bunzow, J. R.; Grandy, D. K.; Zhou, Q.; Van Tol, H. M. H. Molecular biology of the dopamine receptors. Eur. J. Pharmacol. Mol. Pharmacol. Sect. 207:227–286; 1991.
8. Claus, A.; Bollen, J.; DeCuyper, H.; Eneman, M.; Malfroid, M.; Peuskens, J.; Heylen, S. Risperidone vs. haloperidol in the treatment of chronic schizophrenic inpatients: A multicenter double-blind comparative study. Acta Psychiatry Scand. 85:295–305; 1992.
9. Costall, B.; Naylor, R. J. Mesolimbic involvement with behavioral effects indicating antipsychotic activity. Eur. J. Pharmacol. 27:46–58; 1974.
10. Costall, B.; Naylor, R. J.; Nohria, V. Climbing behavior induced by apomorphine in mice: A potent model for the detection of neuroleptic activity. Eur. J. Pharmacol. 50:39–50; 1978.
11. Crow, T. J. Molecular pathology of schizophrenia: More than one disease process? Br. Med. J. 280:66–68; 1980.
12. Crow, T. J.; Ferrier, I. N.; Johnstone, E. C. The two syndrome concept and neuroendocrinology of schizophrenia. Psychiatric Clin. N. Am. 9:99–113; 1986.
13. Darmani, N. A.; Martin, B. R.; Pandey, U.; Glennon, R. A. Do functional relationships exist between 5-HT_{1A} and 5-HT_2 receptors? Pharmacol. Biochem. Behav. 36:901–906; 1990.
14. Davis, K. L.; Kahn, R. S.; Grant Ko, M. D.; Davidson, M. Dopamine in schizophrenia: A review and reconceptualization. Am. J. Psychiatry 148:1474–1486; 1991.
15. DeMaio, D. Clozapine, a novel major tranquilizer. Arzneim-Forsch 22:919–923; 1972.
16. Dunn, R. W.; Corbett, R.; Fielding, S. Effects of 5-HT_{1A} receptor agonists and NMDA antagonists in the social interaction test and the elevated plus maze assay. Eur. J. Pharmacol. 169:1–10; 1989.
17. Farde, L.; Hallidin, C.; Stone-Elander, S.; Sedvall, G. Pet analysis of human dopamine receptor subtypes using ^{11}SCH 23390 and ^{11}C-raclopride. Psychopharmacology (Berl.) 92:278–284; 1987.
18. Fuster, J. M. In: The prefrontal cortex, anatomy, physiology and neurobiology of the frontal lobe. New York: Raven Press; 1980.
19. Gardner, C.; Guy, A. A social interaction model of anxiety sensitive to acutely administered benzodiazepines. Drug Dev. Res. 4: 207–216; 1984.
20. Glaser, T.; Rath, M.; Traber, J.; Zilles, K.; Schleicher, A. Autoradiographic identification and topographical analysis of high affinity serotonin receptor subtypes as a target for the novel putative anxiolytic TVQ 7821. Brain Res. 358:129–134; 1985.
21. Hashimoto, T.; Nishino, N.; Nakai, H.; Tanaka, C. Increase in serotonin 5-HT_{1A} receptors in prefrontal and temporal cortices of brains from patients with chronic schizophrenia. Life Sci. 48:355–363; 1991.
22. Hornykiewicz, O. Brain dopamine in Parkinson's disease and other neurological disturbances. In: Horn, A. S.; Korf, J.; Westernik, B. M. C., eds. The neurobiology of dopamine. London, UK: Academic Press; 1979:633–641.
23. Hyttel, J. SCH 23390 the first selective dopamine D_1 antagonist. Eur. J. Pharmacol. 91:153–154; 1983.
24. Iversen, S. D. Is it possible to model psychotic states in animals? J. Psychopharmacol. 1:154–176; 1987.
25. Jann, M. W. Buspirone: An update on a unique anxiolytic agent. Pharmacotherapy 8:100–116; 1988.
26. Janssen, P. A. J.; Niemegeers, C. J. E.; Aivouters, F.; Schellekens, K. H. L.; Megens, A. A. H. P.; Meert, T. F. Pharmacology of risperidone (R64766), a new antipsychotic with serotonin-S2 and dopamine D_2 antagonist properties. J. Pharmacol. Exp. Ther. 224:685–693; 1988.
27. Kane, J. M.; Honigfeld, G.; Singer, J.; Meltzer, H. Y. Clozapine for the treatment-resistant schizophrenic. Arch. Gen. Psychiatry 45:789–796; 1988.
28. Kane, J. M.; Mayeroff, F. Do negative symptoms respond to pharmacological treatment? Br. J. Psychiatry 155:115–118; 1989.
29. Leysen, J. E.; Gommeren, W.; Eans, A.; DeChaffoy DeCourcelles, D.; Stoof, J. C.; Janssen, P. A. J. Biochemical profile of risperidone a new antipsychotic. J. Pharmacol. Exp. Ther. 247: 661–670; 1988.
30. Leysen, J. E.; Gommeren, W.; Laduron, P. M. A ligand of

choice for neuroleptic receptors. Biochem. Pharmacol. 27:307-328,1977.
31. Litchfield, L.; Wilcoxon, F. A simplified method of evaluating dose effect experiments. J. Pharmacol. Exp. Ther. 96:99-113; 1949.
32. Meltzer, H. Y.; Bastani, B.; Young Kwon, K.; Ramirez, L. F.; Burnett, S.; Sharpe, J. A prospective study of clozapine in treatment-resistant schizophrenic patients. Psychopharmacology (Berl.) 99:568-572; 1989.
33. Meltzer, H. Y.; Burnett, S.; Bastani, B.; Ramirez, L. F. Effects of six months of clozapine treatment on the quality of life of chronic schizophrenic patients. Hospital Commun. Psychiatry 41: 892-897; 1990.
34. Meltzer, H. Y.; Matsubara, S.; Lee, J. Classification of typical and atypical antipsychotic drugs on the basis of dopamine D_1, D_2 and serotonin$_2$ pK_i values. J. Pharmacol. Exp. Ther. 251:238-251; 1989.
35. Mesotten, F.; Suy, E.; Pietquin, M.; Burton, P.; Heylen, S.; Gelders, Y. Therapeutic effect and safety of increasing doses of risperidone (R64744) in psychotic patients. Psychopharmacology (Berl.) 99:445-449; 1989.
36. Peroutka, S. J. Pharmacological differentiation and characterization of 5-HT_{1A}, 5-HT1B and 5-HT1C binding sites in rat frontal cortex. J. Neurochem. 47:29-40; 1986.
37. Peroutka, S. J.; Snyder, S. H. Multiple serotonin receptors: Differential binding of ^{3}H-lysergic acid diethylamide and ^{3}H-spiperone. Mol. Pharmacol. 16:687-699; 1979.
38. Savasta, M.; Dubois, A.; Scatton, B. Autoradiographic localization of D_1 dopamine receptors in the rat brain with [^{3}H] SCH 23390. Brain Res. 375:291-301; 1986.
39. Shopsin, B.; Klein, H.; Aaronsom, M.; Collora, M. Clozapine, chlorpromazine, and placebo in newly hospitalized acutely schizophrenic patients. Arch. Gen. Psychiatry 36:657-664; 1979.
40. Sovner, R.; Parnell-Sovner, N. Use of buspirone in the treatment of schizophrenia. J. Clin. Psychopharmacol. 9:61-62; 1989.
41. Sunahara, R. K.; Guan, H. C.; O'Dowd, B. F.; Seeman, P.; Laurier, L. G.; Ng, G.; George, S. R.; Torchia, J.; Van Tol, H. M. H.; Niznik, H. Y. Cloning the genes for a human dopamine D_5 receptor with higher affinity for dopamine than D_1. Nature 350:614-619; 1991.
42. Szewczak, M. R.; Dunn, R. W.; Corbett, R.; Geyer, H. M.; Rush, D. K.; Wilker, J. C.; Strupczewski, J. T.; Helsley, G. C.; Cornfeldt, M. L. The in vivo pharmacology of the novel antipsychotic HP 873. Soc. Neurosci. Abstr. 16:A109.13; 1990.
43. Van der Maelen, C.; Matheson, G.; Wilderman, R.; Patterson, L. Inhibition of serotonergic dorsal raphe neurons by systemic and iontophoretic administration of buspirone, a nonbenzodiazepine anxiolytic drug. Eur. J. Pharmacol. 129:123-129; 1986.
44. VanTol, H. M. H.; Bunzow, J. R.; Guan, H. C.; Sunahara, R. K.; Seeman, P.; Niznik, H. B.; Civelli, O. Cloning of the gene for a human dopamine D_4 receptor with high affinity for the antipsychotic clozapine. Nature 350:610-614; 1991.
45. Wilmot, C. A.; Szczepanik, A. M.; Brougham, L. R.; Roehr, J. E.; Bores, G.; Hartman, H. B.; Conway, P. G.; Chernack, J.; Strupczewski, J. T.; Glamkowski, E. J.; Ellis, D. B.; Helsley, G. C. The neurochemical profile of HP 873 a potential atypical antipsychotic with D_2/ 5-HT_2 receptor antagonist activity. Soc. Neurosci. Abstr. 16:109.12; 1990.