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Phencyclidine/SKF-10,047 Binding Sites: Evaluation of Function

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McCANN, D. J., R. A. RABIN, S. RENS-DOMIANO AND J. C. WINTER. *Phencyclidine/SKF-10,047 binding sites: Evaluation of function.* PHARMACOL BIOCHEM BEHAV 32(1) 87–94, 1989.—Results of correlation analyses comparing rank-order affinities with rank-order potencies of (+)SKF-10,047, phencyclidine (PCP), and several PCP analogs support the involvement of [^{3}H]-1-[1-(2-thienyl)cyclohexyl]piperidine binding sites (TCP sites) in mediating both the discriminative stimulus properties of PCP and production of 180° perseveration in a 4-arm radial maze. For the same group of drugs, no significant relationship was found to exist between affinities at haloperidol-sensitive (+)[^{3}H]SKF-10,047 binding sites (H-S-SKF sites) and potencies. Also, H-S-SKF sites were found to lack pharmacological selectivity and to be localized in the microsomal fraction of cells. It is concluded that TCP sites may represent receptors which mediate effects not only of PCP, but also of (+)SKF-10,047. In addition, the possibility that H-S-SKF sites may represent a type of membrane-bound enzyme is discussed.

Amiodarone BMY-14,802 Buspirone Drug discrimination Flurazepam Haloperidol Meperidine Microsomal flavin-containing monooxygenase N-dealkylation Phencyclidine Radial maze Receptor Sigma SKF-10,047 Tamoxifen TCP Trihexyphenidyl Tubocurarine

THE psychotomimetic agents phencyclidine (PCP) and N-allyl-N-normetazocine (SKF-10,047) bind with high affinity to at least two common sites in brain tissue, referred to here as "TCP binding sites" (TCP sites) and "Haloperidol-Sensitive SKF-10,047 binding sites" (H-S-SKF sites). TCP sites were first characterized using [^{3}H]PCP (29,31). However, they are now more frequently studied using [^{3}H]-1-[1-(2-thienyl) cyclohexyl]piperidine ([^{3}H]TCP), a radioligand with higher affinity for TCP sites than [^{3}H]PCP (28). H-S-SKF sites, first characterized using (±)[^{3}H]SKF-10,047 in the presence of unlabelled etorphine (20), can be selectively studied using (+)[^{3}H]SKF-10,047 in the presence of unlabelled TCP (6). Only PCP, (+)SKF-10,047, and related psychotomimetic drugs have been shown to bind with high affinity to TCP sites. In contrast, H-S-SKF sites show less pharmacological selectivity; notably, most antipsychotic agents also bind to these sites, with haloperidol demonstrating the highest affinity.

Support for the idea that TCP sites are receptors which mediate certain effects of PCP, SKF-10,047, and related drugs has come from several studies. Notably, significant correlations have been reported between certain measures of potency determined in behavioral tests and affinities at TCP sites (3, 29, 31). Unfortunately, similar correlation analyses have not been performed with respect to affinities at H-S-SKF sites.

In the present investigation, affinities of PCP, (+)SKF-10,047, and six PCP analogs were determined at both TCP sites and H-S-SKF sites. Affinities at each site were then compared, through correlation analyses, with potencies determined in previous drug discrimination studies (1, 16, 17). In addition, potencies for producing 180° perseveration in a 4-arm radial maze were determined for the same group of drugs and correlation analyses were again performed with respect to affinities at each site. Preliminary studies (9) suggested that H-S-SKF sites might play a role in mediating PCP-induced 180° perseveration. Finally, to evaluate the hypothesis that H-S-SKF sites actually represent a type of membrane-bound microsomal enzyme, the pharmacological selectivity and subcellular location of H-S-SKF sites were investigated.

METHOD

Radial Maze Studies

All subjects were male Fischer 344 rats obtained from Charles River Breeding Laboratories, Inc. (Wilmington, MA) at approximately four weeks of age. Subjects were housed in pairs under a natural light-dark cycle and had free access to water in the home cage. They were gradually reduced to 75–80% of their expected free-feeding weight by limiting their access to dry food to two hours per day. Subsequently, they were allowed free access to dry food except during the 24 hours which immediately preceded training or test sessions.

Two 4-arm radial mazes were used in our studies. Their

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design has previously been described in detail (9). Briefly, each maze consists of a central hub, 34 cm in diameter, with four 86 cm by 9 cm arms extending from it. Angles between adjacent arms are 90°. The aluminum walls of each arm are 10 cm high at the center of the maze and slope to a height of 6 cm at the distal end. Pieces of clear plastic extend the height of all walls to 20 cm. An opaque plastic food well is located at the end of each arm.

Data were collected automatically using an IBM Personal Computer, a Lab Linc computer interface system (Coulbourn Instruments, Inc., Lehigh Valley, PA) and photoelectric motion detectors. One motion detector is located at the center of each arm and four are positioned in the central area of each maze. When a rat enters and exits any arm of a maze, the photoelectric beam in that arm is interrupted several times. However, only the first interruption of the beam is recorded as an arm entry; the computer program requires that movement must be detected in the center of the maze before a subsequent arm entry is recorded. Times read from the computer's internal clock are also recorded with each arm entry.

Each subject received training sessions 3 times a day on 2 days each week, Mondays and Thursdays or Tuesdays and Fridays. Although two mazes were utilized, a given subject was trained and subsequently tested in only one of the two mazes. At the start of each session, a subject was placed in the center of the maze with a 45 mg Noyes food pellet located in the food well at the end of each arm. Sessions lasted until all 4 pellets were obtained or for a maximum of 10 minutes. Stable performance was assumed to be present when subjects reached a criterion of no reentries (no entries into previously visited arms) while completing the maze in three consecutive sessions. For 88 subjects used in the present studies (11 groups of 8 subjects), the mean number of sessions required to achieve criterion performance was 6 (range=3 to 14).

After training, subjects continued to perform in the maze 2 days each week, Mondays and Thursdays or Tuesdays and Fridays. Two sessions, similar in design to training sessions, were conducted each day. If a criterion of no reentries was met in the first session, then effects of drug or vehicle injections were evaluated in a test session one hour later. When criterion performance was not achieved in the first session, injections were omitted prior to the second session. Sessions lasted until four arm entries were made or for a maximum of 10 minutes. Effects of drugs on pattern of arm entry were evaluated by a method which treats consecutive movements between opposite arms of the maze as an all-or-none phenomenon, termed "180° perseveration" (9). With the arms of the maze numbered in a clockwise fashion, 180° perseveration was said to occur when one of the following four sequences of arm entries was observed: 1-3-1; 3-1-3; 2-4-2; or 4-2-4. In addition to the incidence of 180° perseveration, rate of arm entry (expressed as arm entries/min) was utilized as a dependent variable. Statistical comparisons of 180° perseveration and rate were performed using the sign test and the Wilcoxon signed-ranks test for paired data, respectively.

Prior to the studies reported here, subjects received various doses of PCP in preliminary studies, with similar doses being administered to subjects in each group. Delineations of the 11 dose-response relationships reported here for individual drugs, one corresponding to each group of 8 subjects trained, were preceded by a 10-day drug-free period. Subsequent studies of drug combinations, in which subjects from 10 of the 11 original groups were used, were also preceded by at least a 10-day drug-free period for each subject. The group of subjects which received (+)-3-PPP was not used in subsequent studies due to resulting convulsions and fatalities.

Binding Studies

Methods for the preparation of membranes, as well as incubation conditions, were those described by Largent *et al.* (6). Brains were removed from male Fischer 344 rats and promptly homogenized in 25 volumes of ice-cold 50 mM Tris HCl (pH 7.7 at 25°C; homogenization buffer). Tissue suspensions were then centrifuged at 45,000×g for 10 min at 4°C. Resulting pellets were resuspended in fresh homogenization buffer and again centrifuged at 45,000×g for 10 min. This procedure was repeated and the final pellets were suspended in 5 mM Tris HCl (pH 8.0 at 25°C; incubation buffer).

All incubations were carried out for 40 minutes at 25°C using a final volume of 0.25 ml. The amount of tissue used was 3.5 mg (original wet weight)/tube for [^{3}H]TCP binding assays and 7.5 mg/tube for (+)[^{3}H]SKF-10,047 binding assays. Time-course experiments revealed that equilibrium binding was attained by 30 min and maintained for at least 60 min with each radioligand. Nonspecific binding of [^{3}H]TCP was defined as binding measured in the presence of 10 μM PCP and nonspecific binding of (+)[^{3}H]SKF-10,047 as that measured in the presence of 100 μm PCP. Saturation experiments were conducted using concentrations of [^{3}H]TCP which ranged from approximately 1 to 100 nM (47.6 Ci/mmole) and concentrations of (+)[^{3}H]SKF-10,047 which ranged from approximately 1 to 1,000 nM (23.0 Ci/mmole from 1 to 20 nM; unlabelled drug was added to 20 nM radioligand to obtain higher concentrations). The two types of PCP/SKF-10,047 binding sites were selectively studied in competition experiments using 2 nM [^{3}H]TCP (TCP sites) or 20 nM (+)[^{3}H]SKF-10,047 in the presence of 500 nM unlabelled TCP (H-S-SKF sites). In all competition experiments, less than 5% of radioligand was bound.

Incubations were stopped by adding 2.5 ml of ice-cold 5 mM Tris HCl (pH 7.7 at 25°C; rinse buffer) followed by vacuum filtration through Whatman GF/B glass fiber filters which had been pretreated with 0.5% polyethylenimine. Filters were then rinsed twice with 5 ml of rinse buffer. Radioactivity retained on the filters was measured by liquid scintillation spectrometry. Efficiency of counting ranged from 36 to 40%.

In [^{3}H]TCP saturation experiments, specific binding to filters alone was subtracted from specific binding to tissue and filters to yield the amount of specific binding to tissue (30). In (+)[^{3}H]SKF-10,047 saturation experiments and in competition experiments, similar corrections were not necessary; specific binding to filters was less than 2% of total specific binding. Data were analyzed using the iterative curve fitting programs EBDA (10) and LIGAND (13). The protein content of membrane preparations ranged from 6 to 8%, based on original wet weight, as measured by the method of Lowry *et al.* (8) using bovine serum albumin (fraction V) as a standard.

Subcellular Localization

Synaptosomal and microsomal fractions were prepared according to the method of Gray and Whittaker (5). Brains from male Fischer 344 rats were homogenized in 10 volumes of 0.32 M sucrose using a glass/teflon motorized homogenizer and the homogenate was centrifuged at 1000×g for 10 min at 4°C. The resulting supernatant was reserved on ice and the pellet was washed by resuspension in 0.32 M sucrose followed by centrifugation at 1000×g for 10 min at 4°C. The

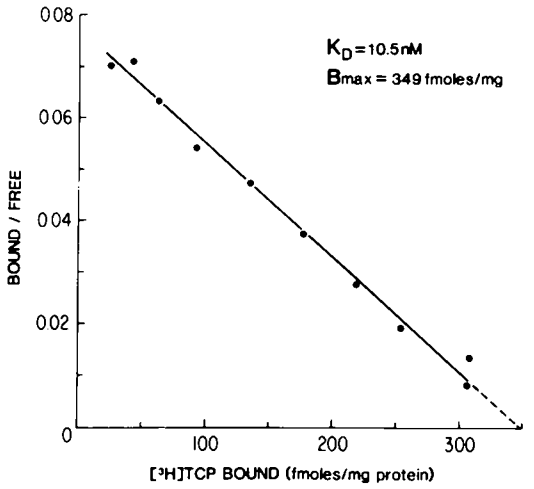

FIG. 1. Scatchard plot of [³H]TCP specific binding to rat brain membranes. Data are from a representative experiment and individual values represent means of triplicate determinations. Concentrations of radioligand ranged from 0.88 to 88 nM. Nonspecific binding was defined using 10 μM PCP.

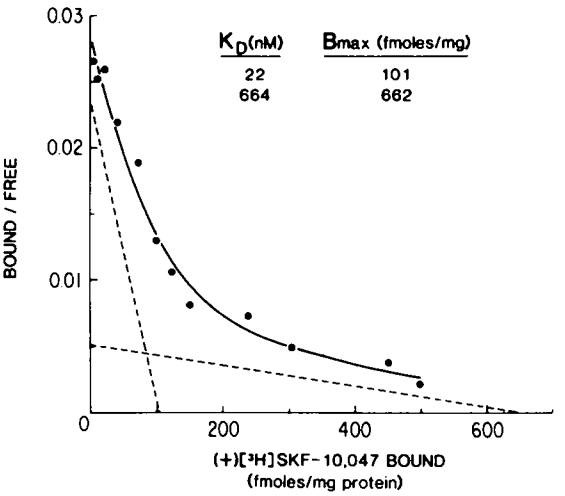

FIG. 2. Scatchard plot of (+)[³H]SKF-10,047 specific binding to rat brain tissue. Data are from a representative experiment and individual values represent means of duplicate determinations. Concentrations of radioligand ranged from 1.1 to 1,200 nM. Nonspecific binding was defined using 100 μM PCP. Dashed lines represent the high and low affinity components of binding.

washed pellet (nuclei, myelin, and tissue debris) was discarded and the combined supernatants were then centrifuged at 20,000×g for 30 min at 4°C. The resulting supernatant was reserved on ice and the pellet was washed once by resuspension in 0.32 M sucrose followed by centrifugation at 20,000×g for 30 min at 4°C. The washed pellet was resuspended in 0.32 M sucrose and layered onto a discontinuous gradient of 0.8 and 1.2 M sucrose. After centrifugation at 113,000×g for 70 min at 4°C, synaptosomes were obtained from the material that collected at the 0.8/1.2 M sucrose interface. Microsomes were obtained from the combined 20,000×g supernatants by centrifugation at 100,000×g for 60 min at 4°C.

Incubations of 20 nM (+)[³H]SKF-10,047 and 2 nM [³H]TCP with synaptosomes and microsomes were carried out as described above. In each assay, the amount of tissue used was such that less than 5% of radioligand was bound. Haloperidol-sensitive (+)[³H]SKF-10,047 binding was defined as the difference between total binding and that measured in the presence of 10 μM haloperidol. Specific binding of [³H]TCP was defined as the difference between total binding and that measured in the presence of 10 μM PCP. Cytochrome *c* reductase activity, a microsomal marker, was measured at room temperature by the spectrophotometric method of Phillips and Langdon (14).

Drugs

Drugs were obtained from the following sources: phencyclidine HCl (PCP), 1-[1-(2-thienyl)cyclohexyl]piperidine HCl (TCP), 1-[1-(2-thienyl)cyclohexyl]pyrrolidine HCl (TCPY), 1-phenylcyclohexylamine HCl (PCA), N-ethyl-1-phenylcyclohexylamine HCl (PCE), N,N-dimethyl-1-phenylcyclohexylamine HCl (PCDMA), 1-[1-(2-thienyl)cyclohexyl]morpholine HCl (TCM), 1-(1-phenylcyclohexyl)morpholine HCl (PCM), and (+)-N-allyl-N-normetazocine HCl [(+)SKF-10,047] from the National Institute on Drug Abuse, Rockville, MD; (±)BMY-14,802 HCl and buspirone HCl from Bristol-Myers Company, Wallingford, CT; amiodarone HCl from Wyeth Laboratories, Philadelphia, PA; flurazepam HCl from Roche Laboratories, Nutley, NJ; haloperidol from McNeil Laboratories, Fort Washington, PA; meperidine HCl from Sterling-Winthrop Research Institute, Rensselaer, NY; tamoxifen citrate from Stuart Pharmaceuticals, Wilmington, DE; trihexyphenidyl HCl from Lederle Laboratories, Pearl River, NY: d-tubocurarine HCl from Sigma Chemical Company, St. Louis, MO; (+)-N-n-propyl-3-(3-hydroxyphenyl)piperidine HCl [(+)-3-PPP] from Research Biochemicals Inc., Wayland, MA; and [³H]TCP (47.6 Ci/mmole) and (+)[³H]SKF-10,047 (23.0 Ci/mmole) from NEN Research Products, Boston, MA. In behavioral studies, lactic acid (0.85%) was used as vehicle for haloperidol and (±)BMY-14,802. Bacteriostatic water for injection served as vehicle for all other drugs.

RESULTS

Scatchard plots of the equilibrium saturation binding of [³H]TCP to rat brain membranes were linear (e.g., Fig. 1), consonant with the labelling of a single class of binding sites. In four such experiments, analysis of [³H]TCP binding yielded a K_d of 10.6±0.3 nM (B_{max}=336±11 fmoles/mg protein). These results are in agreement with previous studies of [³H]TCP binding; K_d values ranging from 7.4 nM (28) to 18 nM (2) have been reported.

Scatchard plots of the equilibrium saturation binding of (+)[³H]SKF-10,047 to rat brain membranes were clearly nonlinear (e.g., Fig. 2). The best fit of data was obtained with a two site model, with resolution into a site with a K_d of 18.5±2.9 nM (B_{max}=90.1±13.5 fmoles/mg protein) and a site with a K_d of 812±373 nM (B_{max}=732±35 fmoles/mg protein) (N=3). In agreement with the present results, previous data from (+)[³H]SKF-10,047 equilibrium saturation experiments have been resolved into a high affinity site with a K_d ranging from 3.6 to 45 nM and a low affinity site with a K_d ranging from 153 to 1,100 nM (6,18).

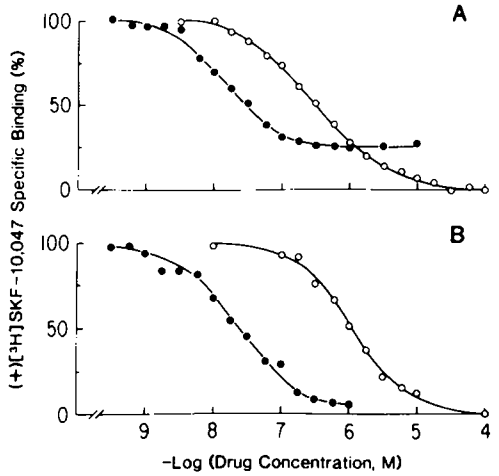

FIG. 3. Competition plots of (+)[³H]SKF-10,047 specific binding to rat brain membranes. Various concentrations of haloperidol (closed circles) or PCP (open circles) were incubated with 20 nM (+)[³H]SKF-10,047 in the absence (panel A) or presence (panel B) of 500 nM unlabelled TCP. Values represent means of 2 (panel A) or 3 (panel B) experiments, each performed utilizing duplicate determinations.

In Fig. 3, panel A are seen the results of competition experiments in which 20 nM (+)[³H]SKF-10,047 was incubated with various concentrations of haloperidol (closed circles) or PCP (open circles). While approximately 75% of (+)[³H]SKF-10,047 specific binding was sensitive to haloperidol, the remainder was insensitive. This haloperidol-insensitive binding can be masked using unlabelled TCP [(6) and Fig. 3, panel B]. Therefore, the affinities of unlabelled drugs for H-S-SKF sites were determined in the presence of 500 nM TCP (Table 1).

The eight PCP-like drugs examined in the competitive binding experiments (Table 1) have been evaluated in previous drug discrimination studies in terms of potencies for causing generalization in PCP-trained rats (1, 16, 17). Results from these studies may be directly compared because procedures for training and testing subjects, as well as methods of data analysis, were identical. Briefly, rats were trained to discriminate between saline and 3.0 mg/kg of PCP in a two-choice avoidance procedure. Because the reported relative potencies were expressed in terms of the number of milligrams of PCP equivalent to 1 mg of each drug, corrections to allow for differences in molecular weight were required; the resulting mole-based relative potencies were 4.83 (PCE), 1.00 (PCP), 0.84 (TCPY), 0.48 [(+)SKF-10,047], 0.43 (PCA), 0.42 (PCDMA), 0.10 (PCM), and 0.07 (TCM).

To facilitate correlation analyses, each drug was assigned a rank of 1 to 8, ranging from the most potent drug (PCE) to the least potent drug (TCM), according to the above mole-based relative potencies. The eight drugs were similarly ranked in terms of their affinities at each of the two types of PCP/SKF-10,047 binding sites (Table 1), with a rank of 1 corresponding to the drug with the highest affinity and a rank of 8 corresponding to the drug with the lowest affinity. As indicated in Fig. 4, panel A, a correlation analysis revealed a significant relationship between affinities at TCP sites and potencies from tests of PCP generalization (r_s=0.88; p<0.01). On the other hand, no significant relationship was found between affinities at H-S-SKF sites and this same measure of behavioral potency (Fig. 4, panel B; r_s=0.57; p>0.05).

TABLE 1

AFFINITIES OF UNLABELLED DRUGS FOR [³H]TCP AND HALOPERIDOL-SENSITIVE (+)[³H]SKF-10,047 BINDING SITES

	IC_{50} (nM)	
	TCP Site	H-S-SKF Site
PCP-Like Drugs		
TCPY	43.6 ± 1.2	433 ± 64
PCP	54.6 ± 2.6	1,090 ± 100
PCE	77.6 ± 8.4	2,700 ± 200
(+)SKF-10,047	326 ± 19	121 ± 21
PCA	636 ± 12	23,500 ± 4000
PCDMA	665 ± 31	1,410 ± 40
TCM	1100 ± 160	68,600 ± 9600
PCM	1510 ± 240	12,700 ± 2800
Dissimilar Tertiary Amines		
Trihexyphenidyl	>10,000	77.3 ± 8.7
Haloperidol	>100,000	40.5 ± 21.5
(+)-3-PPP	>100,000	69.3 ± 32.8
Buspirone	>100,000	237 ± 24
Amiodarone	>100,000	281 ± 63
(±)BMY-14,802	>100,000	440 ± 86
Meperidine	>100,000	689 ± 61
Flurazepam	>100,000	1,550 ± 230
Tamoxifen	>100,000	5,620 ± 680

Membranes from rat brain were incubated with 2 nM [³H]TCP ("TCP sites") or 20 nM (+)[³H]SKF-10,047 in the presence of 500 nM TCP ("H-S-SKF sites") and various concentrations of unlabelled drug. Values represent means and standard errors for 3 or more determinations.

In Fig. 5 are seen dose response relationships for (+)SKF-10,047, PCP and the various PCP analogs with regard to production of 180° perseveration in a 4-arm radial maze. The apparent rank order of potency is PCE > TCPY > PCP > (+)SKF-10,047 > PCA > PCDMA > TCM > PCM. Based on these results, each drug was again assigned a rank of 1 to 8, ranging from the most potent drug to the least potent drug. Correlation analyses were again performed and, as indicated in Fig. 6 (panel A), a significant relationship was found to exist between affinities at TCP sites and potencies measured in the 4-arm radial maze (r_s=0.93; p<0.01). Again, no significant relationship was found with affinities at H-S-SKF sites (Fig. 6, panel B; r_s=0.55; p>0.05).

Because haloperidol, (±)BMY-14,802, and (+)-3-PPP demonstrate a high affinity for H-S-SKF sites and virtually no affinity for TCP sites (Table 1), effects of these drugs were evaluated in the 4-arm radial maze. All three drugs failed to cause 180° perseveration (Table 2). For each drug, doses were increased until all subjects failed to perform in the maze. At the highest doses of haloperidol and (±)BMY-14,802 examined, performance was precluded by prominent sedation. The highest dose of (+)-3-PPP tested (64 mg/kg) caused convulsions in all subjects.

Of the three drugs which failed to cause 180° perseveration, (±)BMY-14,802 was chosen for evaluation as a potential PCP antagonist due to its reported selectivity for H-S-SKF sites (27). In a cross-over design, either vehicle or

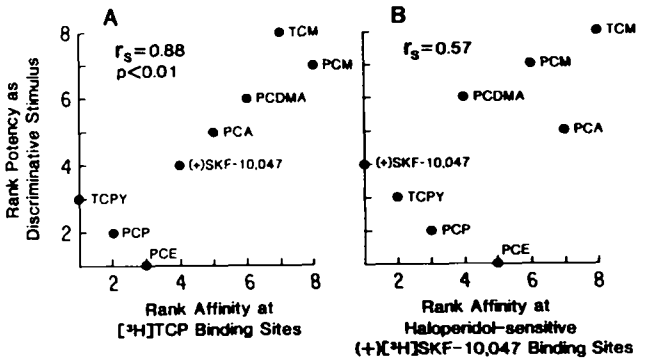

FIG. 4. Spearman rank correlation analyses of potencies from tests of PCP generalization and affinities at TCP sites (panel A) or H-S-SKF sites (panel B) for (+)SKF-10,047, PCP, and 6 PCP analogs. Drugs were ranked from 1 to 8 according to their mole-based relative potency values [calculated from the results of Shannon and co-workers (1, 16, 17)] and the IC_{50} values shown in Table 1; ranks of 1 correspond to the highest potency or affinity and ranks of 8 correspond to the lowest potency or affinity.

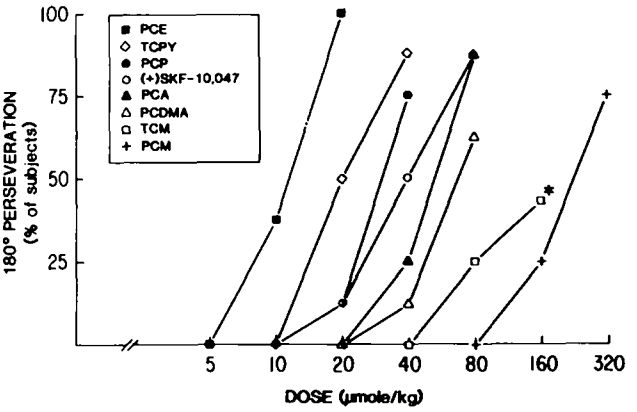

FIG. 5. Dose-response relationships for PCP-like drugs with regard to production of 180° perseveration in a 4-arm radial maze. Eight groups of 8 subjects received increasing doses of a given drug until greater than 50% of subjects demonstrated 180° perseveration or until failure to complete sessions was observed. All drugs were injected IP 15 min before test sessions. In 128 vehicle test sessions, one given to each subject prior to and after dose-response determinations, 180° perseveration was observed only once. *Value represents only 7 of 8 subjects.

a given dose of (±)BMY-14,802 (IP) was administered to groups of 20 subjects 15 minutes after 10 mg/kg of PCP (IP, 30 minutes before test sessions). At doses of 1, 2, and 4 mg/kg, (±)BMY-14,802 failed to significantly alter either the rate of arm entry or the occurrence of 180° perseveration. However, 8 mg/kg of (±)BMY-14,802 caused significant decreases in both variables; rate was reduced from 5.6 to 4.0 arms/min ($p<0.05$) and the incidence of 180° perseveration was reduced from 95 to 60% ($p<0.05$). When a higher dose of (±)BMY-14,802 (16 mg/kg) was combined with PCP, subjects failed to perform in the maze. That the attenuation of PCP-induced 180° perseveration by (±)BMY-14,802 may not reflect pharmacological antagonism is suggested by its concomitant rate depressing effects.

In theory, decreased rate per se or a combination of decreased rate and increased ataxia may lead to a lower incidence of PCP-induced 180° perseveration by increasing the probability of turning in the central area of the maze. To evaluate this possibility, interactions of d-tubocurarine and PCP were examined. In preliminary experiments, doses of 0.04 and 0.08 mg/kg of d-tubocurarine (IP, 15 minutes before test sessions) were found to significantly decrease rates of arm entry and cause noticeable ataxia in the maze. In Table 3 are seen the effects of vehicle or d-tubocurarine when administered 15 minutes after a 10 mg/kg dose of PCP. At 0.08 mg/kg, a dose of d-tubocurarine which significantly decreased rate of arm entry, a concomitant significant decrease in PCP-induced 180° perseveration was observed. Because d-tubocurarine is unable to cross the blood-brain barrier, its pharmacological effects are generally assumed to arise outside the central nervous system. Therefore, the present results strongly suggest that ataxia and decreased rate lead to an increase in the probability of turning in the central area of the maze.

Because results of correlation analyses (Figs. 4 and 6) failed to support a role for H-S-SKF sites in mediating effects of PCP and (+)SKF-10,047, we began to consider what function these sites might serve other than as receptors. Based on their high concentration in membranes from rat liver (15), the possibility that H-S-SKF sites actually represent a type of membrane-bound enzyme was considered.

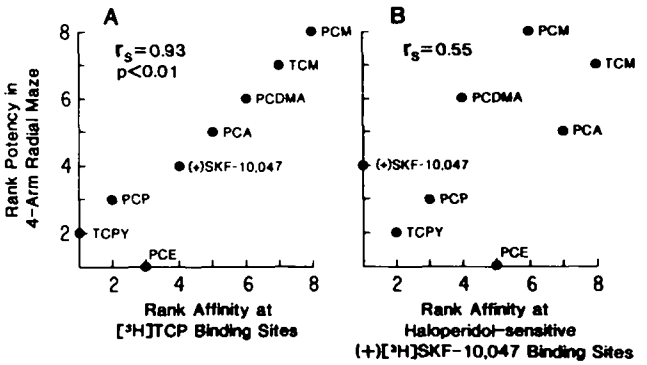

FIG. 6. Spearman rank correlation analyses of potencies for causing 180° perseveration in a 4-arm radial maze and affinities at TCP sites (panel A) or H-S-SKF sites (panel B) for (+)SKF-10,047, PCP, and 6 PCP analogs. Drugs were ranked from 1 to 8 according to the relative potencies apparent from Fig. 5 and the IC_{50} values shown in Table 1; ranks of 1 correspond to the highest potency or affinity and ranks of 8 correspond to the lowest potency or affinity.

Because the primary metabolic fate of haloperidol, the drug showing the highest affinity for H-S-SKF sites, is N-dealkylation (19), six additional drugs were chosen for examination in binding studies: amiodarone, buspirone, flurazepam, meperidine, tamoxifen, and trihexyphenidyl. Except for trihexyphenidyl, all have been shown to undergo N-dealkylation. Trihexyphenidyl, while not known to be a substrate for N-dealkylation, was chosen for study because of the tertiary nitrogen atom contained in its piperidine ring. As a group, these drugs exert a range of diverse pharmacological actions, ranging from antiarrhythmic to antineoplastic. Clinical experience with these drugs suggest that they are neither PCP-like nor antipsychotic in nature. However, as seen in Table 1, each drug demonstrates a high affinity for H-S-SKF sites. The affinities of flurazepam and

TABLE 2

DRUGS FAILING TO CAUSE 180° PERSEVERATION IN THE 4-ARM RADIAL MAZE

Treatment and Dose (mg/kg)	Session Ratio	Rate (arms/min)	180° Perseveration (% of subjects)
Haloperidol			
0	48/48	3.8	0
0.025	8/8	3.0	0
0.05	6/8	2.9	0
0.1	7/8	2.8	0
0.2	4/8	1.1*	0
0.4	2/8	0.5*	0
0.8	0/8	0.0†	—
(±)BMY-14,802			
0	40/40	4.3	0
1	8/8	5.6	0
2	8/8	4.3	0
4	7/8	2.9	0
8	3/8	0.8†	0
16	0/8	0.2†	—
(+)-3-PPP			
0	56/56	5.0	0
0.5	8/8	5.1	0
1	8/8	4.7	0
2	8/8	3.6†	0
4	8/8	3.3	0
8	8/8	3.9	0
16	8/8	3.9	0
32	8/8	3.6	0

Three groups of 8 rats received increasing doses of haloperidol, (±)BMY-14,802, or (+)-3-PPP, each dose being counterbalanced with a vehicle test session. All drugs were injected IP 15 min before test sessions. Session ratios refer to the fraction of sessions completed (i.e., in which 4 arm entries were made). For (+)-3-PPP, a dose of 64 mg/kg was found to cause convulsions and death. *$p<0.05$, †$p<0.01$.

TABLE 3

INTERACTIONS OF d-TUBOCURARINE AND PCP IN THE 4-ARM RADIAL MAZE

Dose (mg/kg) PCP	d-Tubocurarine	Session Ratio	Rate (arms/min)	180° Perseveration (% of subjects)
10	0	72/72	4.2	83
10	0.02	24/24	4.6	83
10	0.04	24/24	4.7	92
10	0.08	24/24	1.8*	25*
0	0	144/144	6.0	0

Various doses of d-tubocurarine or vehicle were combined with 10 mg/kg of PCP in a cross-over design. Vehicle/vehicle sessions were conducted before and after each pair of drug treatments to provide an index of baseline stability. PCP and d-tubocurarine were injected IP 30 and 15 min before test sessions, respectively. Session ratios refer to the fraction of sessions completed (i.e., in which 4 arm entries were made). *$p<0.005$.

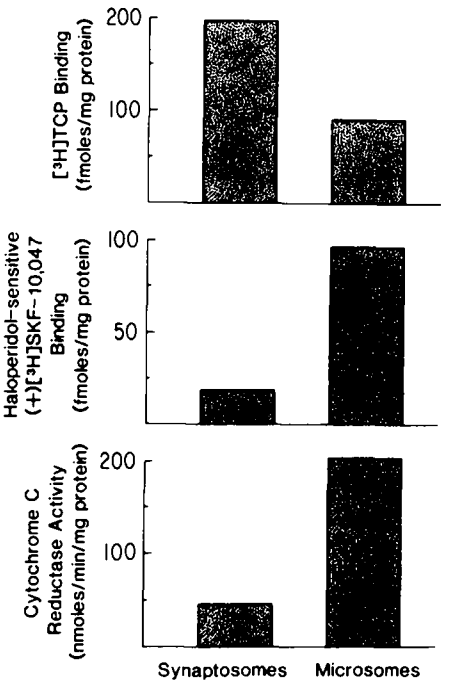

FIG. 7. Comparison of [³H]TCP and haloperidol-sensitive (+)[³H]SKF-10,047 specific binding with cytochrome *c* reductase activity, a microsomal marker, in rat brain synaptosomes and microsomes. Data are from a representative experiment in which 2 nM [³H]TCP and 20 nM (+)[³H]SKF-10,047 were utilized and nonspecific binding was defined using 10 μM PCP or haloperidol, respectively.

tamoxifen at H-S-SKF sites, while slightly lower than that of PCP, are approximately in the middle of the range of affinities exhibited by the behaviorally active PCP analogs. The affinities of amiodarone, buspirone, meperidine and trihexyphenidyl at H-S-SKF sites are all greater than that of PCP and, in most cases, are comparable to that of (+)SKF-10,047. In sharp contrast, only (+)SKF-10,047, PCP, and the various PCP analogs demonstrate a high affinity at TCP sites.

In Fig. 7 are seen data from a representative experiment in which the subcellular localization of H-S-SKF sites was examined. Current technology for subcellular fractionation does not permit a total separation of microsomal membranes from synaptosomal membranes. Therefore, cytochrome *c* reductase activity was used as a microsomal marker and, because TCP sites are concentrated in synaptosomes (29), [³H]TCP binding was used as a synaptosomal marker. Haloperidol-sensitive (+)[³H]SKF-10,047 binding clearly followed cytochrome *c* reductase activity. For two such experiments, mean synaptosome/microsome ratios for [³H]TCP binding, haloperidol-sensitive (+)[³H]SKF-10,047 binding, and cytochrome *c* reductase activity were 3.0, 0.12, and 0.15, respectively.

DISCUSSION

Because TCP sites were originally characterized using [³H]PCP (29,31), they have been termed "PCP receptors." Because H-S-SKF sites were originally characterized using [³H]SKF-10,047 (20), which is the prototypical σ-opioid agonist (11), they have been termed "σ-receptors." The present results support the hypothesis that TCP sites are

receptors which mediate certain behavioral effects not only of PCP, but also of SKF-10,047. On the other hand, data pertaining to H-S-SKF sites suggest that they may represent a form of microsomal enzyme.

Results of our correlation analyses clearly support the involvement of TCP sites in mediating both the discriminative stimulus properties of PCP and production of 180° perseveration in a 4-arm radial maze. Using a slightly different group of drugs, Zukin and Zukin (31) previously demonstrated a significant correlation between affinities at TCP sites and potencies in tests of PCP generalization. In addition, several other measures of potency have been significantly correlated with affinities at TCP sites: production of ataxia in mice (29,31), production of catalepsy in pigeons (12), and production of stereotyped behavior in rats (3). In contrast, comparisons of potencies with affinities at H-S-SKF sites have not previously been reported. The present results fail to support the involvement of H-S-SKF sites in mediating effects of PCP, (+)SKF-10,047, and related drugs.

Formation of the hypothesis that H-S-SKF sites actually represent a type of membrane-bound enzyme was based on their high concentration in membrane homogenates derived from rat liver (15), as well as the lack of evidence supporting their functionality as receptors. While the B_{max} of (±)[^{3}H]SKF-10,047 binding to rat liver membranes was reported to be 9,300 fmoles/mg protein, that reported by Su (20) for binding to guinea pig brain membranes was only 663 fmoles/mg protein. This difference is especially interesting in light of the fact that H-S-SKF sites are more concentrated in guinea pig brain than in rat brain. To provide a direct comparison of rat liver and rat brain, membranes from each tissue were incubated with 20 nM (+)[^{3}H]SKF-10,047 in the presence and absence of 10 μM haloperidol. Resulting amounts of haloperidol-sensitive binding were 120,547± 3,592 and 3,102±42 DPM/mg protein for liver and brain, respectively (N=3 for each; McCann and Rabin, unpublished). Pharmacological characterization by Somovilova *et al.* (15) revealed that H-S-SKF sites in rat liver are similar to those previously characterized in brain tissue; the rank order of affinities observed using liver membranes was haloperidol (IC_{50}=15 nM) > (±)SKF-10,047 (154 nM) > (±)propranolol (290 nM) ≈ imipramine (316 nM) > PCP (1,300 nM).

In rats the primary metabolic fate of haloperidol, the drug which demonstrates the highest affinity at H-S-SKF sites, is N-dealkylation (19). Interestingly, nearly all drugs previously reported to bind with high affinity to H-S-SKF sites are secondary or tertiary amines which are known to undergo N-dealkylation. In addition, many of these drugs are not psychotomimetic or antipsychotic in nature; for example, imipramine, labetalol, phenoxybenzamine, propranolol, pyrilamine, sufentanil, and tripelennamine all demonstrate affinities for H-S-SKF sites which lie within the range of (+)SKF-10,047 and PCP (20, 21, 23, 24). From the present study, the high affinities of amiodarone, buspirone, flurazepam, meperidine, tamoxifen, and trihexyphenidyl at H-S-SKF sites lend support to the hypothesis that these binding sites actually represent a type of membrane-bound enzyme, possibly one which participates in the process of N-dealkylation. Recently, Su *et al.* (22) reported that progesterone binds with high affinity to H-S-SKF sites. While progesterone is not a substrate for N-dealkylation, it may act as a noncompetitive inhibitor of the enzyme in question. The idea that H-S-SKF sites are receptors which mediate psychotomimetic and antipsychotic effects of drugs seems irreconcilable with the observed lack of pharmacological selectivity.

The specific enzyme responsible for N-dealkylation of haloperidol has not been determined. However, only two types of enzymes are thought to participate in N-oxidation processes: forms of microsomal flavin-containing monooxygenase (MFCMO) and cytochrome P-450-dependent mixed function oxidase (MFO). Gorrod (4) has suggested a method for predicting the extent to which these two types of enzymes act on a nitrogen-containing compound based on its structure and pK_a. According to this method, haloperidol, a basic tertiary amine (pK_a=8.2 to 8.3), would be predicted to serve as a substrate for only MFCMO and N-dealkylation would, therefore, be expected to occur subsequent to MFCMO-mediated N-oxide formation.

While both MFCMO and MFO are microsomal, plasma membrane-bound receptors are generally concentrated in the synaptosomal fraction of cells. Vincent *et al.* (29), using [^{3}H]PCP as a radioligand, have previously demonstrated that TCP sites are concentrated in the synaptosomal fraction of cells and the [^{3}H]TCP binding data in Fig. 7 are in agreement with this finding. However, (+)[^{3}H]SKF-10,047 binding data clearly indicate that H-S-SKF sites are microsomal. These results are not compatible with the idea that H-S-SKF sites are synaptic receptors. Instead, they suggest that H-S-SKF sites may represent a type of microsomal membrane-bound enzyme.

Recently, Tam *et al.* (26) have reported that haloperidol antagonizes certain effects of PCP and (+)SKF-10,047 on visually-evoked potentials. These results were interpreted as evidence that H-S-SKF sites are receptors which mediate psychotomimetic and antipsychotic effects of drugs. However, the observed effects of PCP and (+)SKF-10,047 may have involved activation of dopaminergic neurons and the likely involvement of dopamine receptors in the effects of haloperidol was not ruled out. Results of studies examining the stimulus properties of (+)SKF-10,047 (25,26) have also been interpreted as evidence that H-S-SKF sites represent psychotomimetic/antipyschotic receptors. However, generalization to clonidine and antagonism by yohimbine (25) would argue against this; clonidine is not psychotomimetic and yohimbine is not antipsychotic.

In conclusion, there exists substantial evidence supporting a role for TCP sites as receptors which mediate certain effects of PCP and (+)SKF-10,047. In addition to the behavioral results previously mentioned, results of recent electrophysiological studies suggest that TCP sites play an important role in modulating the function of ion channels associated with certain glutamate receptors [see (7) for review]. On the other hand, H-S-SKF sites appear to lack the pharmacological selectivity which would be expected of a receptor. In addition, their microsomal localization, as well as their high concentration in liver tissue, suggests that H-S-SKF sites may represent a type of membrane-bound enzyme. The most likely candidate for this enzyme would appear to be a form of MFCMO. However, further studies of H-S-SKF sites are clearly needed; their functionality has not been demonstrated as receptors or enzymes. In addition, the possibility that H-S-SKF sites may be located on nonsynaptic areas of plasma membrane cannot be ruled out based on the present data.

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NOTE ADDED IN PROOF

Recent reports have suggested the involvement of H-S-SKF sites in mediating certain motor effects of antipsychotic drugs (Walker *et al.*, Neurology 38:961–965; 1988) as well as certain effects of "sigma" drugs on phosphoinositide metabolism (Bowen *et al.*, Eur. J. Pharmacol. 149:399–400; 1988). We wish to note that the reported findings are reconcilable with the hypothesis, proposed here, that H-S-SKF sites represent a type of membrane-bound enzyme. An important commonality of the two recent studies is that haloperidol was observed to cause (+)SKF-10,047-like effects. In fact, all "sigma" drugs tested produced similar effects. Thus, the observed effects may have been mediated through the inhibition of an enzyme's normal function.

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