Differentiation of [^{3}H]phencyclidine and (+)-[^{3}H]SKF-10,047 binding sites in rat cerebral cortex

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The potency of a series of opioid and non-opioid psychotomimetic drugs to inhibit the specific binding of [^{3}H]PCP and (+)-[^{3}H]SKF-10,047 to rat cerebral cortical membranes was examined. (+)-PCMP, the 3-methylpiperidino analog of PCP, was a potent inhibitor of the specific binding of both ligands. All of the other 12 compounds examined, however, displayed a 3–277-fold selectivity for either [^{3}H]PCP or (+)-[^{3}H]SKF-10,047 binding. These results suggest that although these opioid and non-opioid psychotomimetics bind to both sites, most have significantly different affinities. The binding sites for [^{3}H]PCP appear to be distinct from the 'sigma' binding sites labeled with (+)-[^{3}H]SKF-10,047.

SKF-10,047 *Sigma receptor* *Phencyclidine* *Phencyclidine receptor* *Psychotomimetic activity*

1. INTRODUCTION

Psychotomimetic behavior may be induced in man by many drugs including phencyclidine (PCP) and the benzomorphans *N*-allylnormetazocine (SKF-10,047), cyclazocine and pentazocine. Attempts to delineate the neurochemical mechanism of action of psychotomimetic drugs has focused on their interactions with specific central nervous system (CNS) receptors. Using the chronic spinal dog preparation, racemic SKF-10,047 was the prototypic agonist for identifying the sigma opioid receptor [1]. PCP has been shown to produce similar pharmacological actions to SKF-10,047 when tested in this preparation [2]. More recently, receptor binding studies using radiolabeled psychotomimetic drugs have been employed to investigate their site(s) of action. Using these procedures, specific CNS binding sites for [^{3}H]PCP and (+)-[^{3}H]SKF-10,047 have been demonstrated [3–8]. Although it is not clear if the (+)-[^{3}H]SKF-10,047 binding site is structurally related to the classical sigma opioid receptor, the question remains as to whether the psychotomimetic benzomorphans bind to the same site in the CNS as the PCP-like compounds. Behavioral evidence and some receptor binding studies suggest that these sites are identical [3–5,9–11], however, other receptor binding and neurochemical studies suggest that these sites are distinct [6,7,12,13]. To address this issue, we compared the relative potencies of several benzomorphans, PCP analogs and butaclamol enantiomers for inhibiting the binding of (+)-[^{3}H]SKF-10,047 and [^{3}H]PCP to rat cerebral cortical membranes. (−)-Butaclamol has been shown to inhibit potently the binding of (+)-[^{3}H]SKF-10,047, however, its effects on the [^{3}H]PCP site have not been reported [7]. With the exception of (+)-PCMP which showed similar potencies for both sites, all of the other compounds were more potent inhibitors of binding of either (+)-[^{3}H]SKF-10,047 or [^{3}H]PCP. Thus, although some PCP derivatives are capable of binding to the 'sigma' receptor (as defined by (+)-[^{3}H]SKF-10,047) at low concentrations (and vice versa) these 2 recognition sites appear to be pharmacologically distinct.

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2. MATERIALS AND METHODS

Cerebral cortices from adult male Sprague-Dawley rats (150–200 g) were dissected on ice, pooled and homogenized in 25 vols (w/v) of 50 mM Tris-HCl buffer (pH 7.7 at 22°C) using a Polytron (Brinkmann, Westbury, NY, PT 10 probe, 15 s). The homogenate was centrifuged at 37000 × *g* and the pellet resuspended in the original volume of Tris buffer. This procedure was repeated twice and the final pellet resuspended in 8 vols of 50 mM Tris-HCl buffer (pH 7.7, 22°C). 100-μl aliquots of tissue (approx. 1 mg protein) were added to 0.9 ml distilled/deionized water (i.e. final Tris concentration = 5 mM) containing (+)-[^{3}H]SKF-10,047 (2 nM, spec. act. 43.3 Ci/mmol, NEN, Boston, MA) or [^{3}H]PCP (4 nM, spec. act. 49.9 Ci/mmol, NEN) in the presence or absence of drugs. Non-radioactive SKF-10,047 (10 μM) or PCP (10 μM) was used to define non-specific binding in the respective binding assay. Incubations for the (+)-[^{3}H]SKF-10,047 or [^{3}H]PCP assays were carried out at 22°C for 45 min or 4°C for 60 min, respectively (cf. [4,6]). At the completion of the incubation, the assay was terminated by rapid filtration using a cell harvester (Brandel, Gaithersburg, MD) over Whatman GF/B or GF/C filters that were presoaked in 0.5% polyethyleneimine to reduce nonspecific filter binding of radioligand. The filters were washed 3 times with 4 ml ice-cold 5 mM Tris-HCl buffer (pH 7.7). Under these conditions, there was no significant displacement of the radioactive ligands from the filters by non-radioactive drugs. The filters were placed in 10 ml Readi-Solv MP (Beckman) and the radioactivity quantified by conventional liquid scintillation spectroscopy. Specific binding, defined as the difference between total and nonspecific binding, was approx. 77% for [^{3}H]PCP and 50% for (+)-[^{3}H]SKF-10,047.

SKF-10,047, PCP, *N*-[1-(2-thienyl)cyclohexyl]piperidine (TCP) and *N*-ethyl-1-phenylcyclohexylamine (PCE) were supplied by Dr Richard Hawks (NIDA). The following compounds were synthesized at NIH: 1-(1-phenylcyclohexyl)-3-methylpiperidine (PCMP) and 4-fluoro-1-[1-(2-thienyl)cyclohexyl]piperidine (F-TCP). Dexoxadrol and levoxadrol were generously supplied by the Upjohn Co. (Kalamazoo, MI) and pentazocine and (+)-cyclazocine by Sterling Winthrop (Rensselaer, NY). The enantiomers of butaclamol were purchased from Research Biochemicals (Wayland, MA).

3. RESULTS

The (+) enantiomer of PCMP was a potent inhibitor of specific [^{3}H]PCP and (+)-[^{3}H]SKF-10,047 binding to rat cerebral cortical membranes (fig.1A, table 1). The IC_{50} values were 30 ± 5 nM (mean ± SE) and 30 ± 6 nM for [^{3}H]PCP and (+)-[^{3}H]SKF-10,047, respectively (fig.1A, table 1). (+)-PCMP was 6.7-fold more potent than (−)-PCMP at inhibiting (+)-[^{3}H]SKF-10,047 binding and 51.7-fold more potent than (−)-PCMP at inhibiting [^{3}H]PCP binding (fig.1, table 1). There was a significant difference between the potency of (−)-PCMP in inhibiting (+)-[^{3}H]SKF-10,047 vs [^{3}H]PCP binding ($p < 0.01$).

Dexoxadrol and the PCP analogs TCP, F-TCP and PCE were potent inhibitors of [^{3}H]PCP binding but were only weak inhibitors of (+)-[^{3}H]SKF-10,047 binding (table 1). Levoxadrol, the

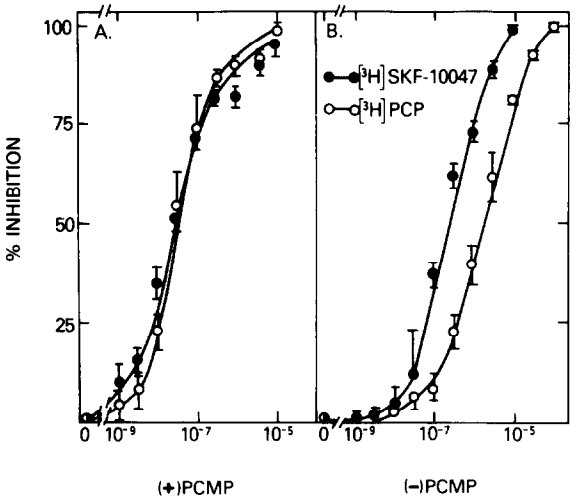

Fig.1. Inhibition of specific (+)-[^{3}H]SKF-10,047 and [^{3}H]PCP binding by PCMP enantiomers. Rat cerebral cortical membranes were incubated in 5 mM Tris buffer with (+)-[^{3}H]SKF-10,047 (●—●) or [^{3}H]PCP (○—○) in the presence or absence of the indicated concentrations of (+)-PCMP (A) or (−)-PCMP (B). Data represent the mean ± SE of 3 separate experiments each carried out in duplicate or triplicate.

Table 1

Relative potencies of PCP and benzomorphan analogs and butaclamol enantiomers for inhibiting specific [^{3}H]PCP and (+)-[^{3}H]SKF-10,047 binding to rat cerebral cortical membranes

Drug	IC_{50} (nM)	
	[^{3}H]PCP	(+)-[^{3}H]SKF-10,047
PCP	40 ± 10	267 ± 33
(+)-PCMP	30 ± 5	30 ± 6
(−)-PCMP	1830 ± 600	200 ± 29
TCP	11 ± 1	2300 ± 700
F-TCP	25 ± 5	2200 ± 1400
PCE	38 ± 6	1300 ± 300
Dexoxadrol	33 ± 2	650 ± 230
Levoxadrol	17000 ± 1600	5300 ± 2300
(+)-SKF-10,047	217 ± 17	13 ± 3
Pentazocine	3600 ± 700	13 ± 1
(+)-Cyclazocine	163 ± 36	35 ± 2
(−)-Butaclamol	>10000	48 ± 21
(+)-Butaclamol	>10000	4000 ± 1000

Values represent the mean ± SE of the IC_{50} values (concentration inhibiting 50% of specific binding) of 3 separate experiments

(−) enantiomer of dexoxadrol, was a weak inhibitor of both ligands. Pentazocine and (+)-cyclazocine, analogs of SKF-10,047, were more potent inhibitors of (+)-[^{3}H]SKF-10,047 binding than [^{3}H]PCP binding. (−)-Butaclamol was 83-fold more potent than (+)-butaclamol as an inhibitor of (+)-[^{3}H]SKF-10,047 binding, however neither enantiomer of butaclamol at concentrations up to 10 μM inhibited [^{3}H]PCP binding. The logarithmic correlation coefficient (Pearson's product moment) between the IC_{50} values of these drugs in inhibiting (+)-[^{3}H]SKF-10,047 and [^{3}H]PCP binding was 0.04 demonstrating that there is no significant correlation between the potencies of the drugs listed in table 1 to inhibit the binding of each ligand.

4. DISCUSSION

This study represents a systematic investigation of the inhibition of both (+)-[^{3}H]SKF-10,047 and [^{3}H]PCP binding by butaclamol enantiomers, benzomorphans and PCP derivatives. The compounds used here were specifically chosen in an attempt to clarify the relationship between these two binding sites. (+)-PCMP, the 3-methylpiperidino analog of PCP, was a potent inhibitor of the specific binding of both ligands to rat cerebral cortical membranes. The inhibition of binding of both radioligands was stereoselective since (−)-PCMP was significantly less potent than (+)-PCMP. (−)-PCMP was more potent as an inhibitor of (+)-[^{3}H]SKF-10,047 binding than [^{3}H]PCP binding. The effects of PCMP enantiomers on (+)-[^{3}H]SKF-10,047 binding have not been previously reported and suggests further that specific (+)-[^{3}H]SKF-10,047 binding is stereoselectively inhibited although the degree of stereoselectivity for (+)-[^{3}H]SKF-10,047 is less than for [^{3}H]PCP binding. The effects of the PCMP enantiomers on [^{3}H]PCP binding is similar to that previously reported by our laboratory [14,15].

In this study, there were relatively large differences in the potencies of most drugs to inhibit [^{3}H]PCP and (+)-[^{3}H]SKF-10,047 binding. The PCP analogs TCP, F-TCP, PCE and dexoxadrol were more potent inhibitors of [^{3}H]PCP binding than (+)-[^{3}H]SKF-10,047 binding. Conversely, the butaclamol enantiomers and benzomorphans, pentazocine and (+)-cyclazocine, were more potent inhibitors of the binding of (+)-[^{3}H]SKF-10,047 than [^{3}H]PCP. The effects of the fluoro derivative of TCP (F-TCP) on [^{3}H]PCP or (+)-[^{3}H]SKF-10,047 binding have not been reported previously. The results of benzomorphans and butaclamol enantiomers on (+)-[^{3}H]SKF-10,047 binding and PCP derivatives on [^{3}H]PCP binding in terms of relative order of potencies are in agreement with previous studies [3,6,8,15]. The effects of butaclamol enantiomers on [^{3}H]PCP binding have not been reported previously. In a recent report, Mendelsohn and co-workers [9] found a significant correlation between the 2 binding sites using certain benzomorphans and PCP analogs. We also found a significant correlation when similar drugs were used. However, extension of the work to include other compounds reduced the correlation coefficient to a statistically insignificant value. Our results, therefore, suggest that (+)-[^{3}H]SKF-10,047 and [^{3}H]PCP binding sites are distinct and support the hypotheses of Tam [6], Martin et al. [8] and Tam and Cook [7]. It is not clear from the present study, however, whether (+)-PCMP binds to a site common to [^{3}H]PCP

and (+)-[^{3}H]SKF-10,047 or if this compound coincidently binds to both sites.

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