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RAPID COMMUNICATION

(−)PPAP: A New and Selective Ligand for Sigma Binding Sites

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GLENNON, R. A., G. BATTAGLIA AND J. D. SMITH. *(−)PPAP: A new and selective ligand for sigma binding sites.* PHARMACOL BIOCHEM BEHAV **37**(3) 557–559, 1990.—Most agents employed for the investigation of sigma (σ) binding sites display relatively low affinity for these sites, bind both at σ sites and at either phencyclidine (PCP) sites or dopamine receptors with similar affinity, and/or produce some dopaminergic activity in vivo. We describe a new agent, (−)PPAP or R(−)-N-(3-phenyl-n-propyl)-1-phenyl-2-aminopropane hydrochloride, that binds with high affinity and selectivity at σ (IC_{50} = 24 nM) versus either PCP sites (IC_{50} >75,000 nM) or D1 and D2 dopamine receptors (IC_{50} >5,000 nM). The σ affinity of this agent is comparable to that of the standard ligands (+)-3-PPP and DTG. Furthermore, although (−)PPAP is structurally related to amphetamine, it neither produces nor antagonizes amphetamine-like stimulus effect in rats trained to discriminate 1 mg/kg of S(+)amphetamine from saline.

Sigma receptor Radioligand binding

UNLIKE most classical opiates, certain benzomorphan derivatives such as cyclazocine, pentazocine, and N-allylnormetazocine (NANM; SKF-10,047) produce psychotomimetic effects in animals and humans. Martin and co-workers (8) proposed the existence of "σ-opiate" receptors to account for the actions of these agents. Because these agents can produce effects that are reminiscent of some of those produced by the psychotomimetic agent phencyclidine (PCP), and because they were found to bind at PCP binding sites, it was once thought that PCP and σ binding sites might be synonymous (i.e., "PCP/σ-sites"). More recent studies, using [^{3}H]S(+)-NANM as a radioligand to label σ sites, have shown that the regional distribution of PCP and σ sites is different [e.g., (4)]. Furthermore, the neuroleptic agent haloperidol, which displays high affinity for [^{3}H]NANM-labeled σ sites, possesses low affinity for PCP sites. In addition, [^{3}H]haloperidol (in the presence of agents to preclude binding to dopamine receptors) is now commonly employed to label σ sites. [For recent reviews, see (2) and (12).]

Among those agents used for studying σ sites, two of the more common are NANM and haloperidol; however, these agents lack selectivity. As mentioned above, NANM and other σ-opiates bind at PCP sites, and haloperidol displays a comparable affinity for dopamine receptors and σ-sites. Two other recently available σ ligands include S(+)3-(3-hydroxyphenyl)-N-(1-propyl)piperidine (3-PPP) and 1,3-di(2-tolyl)guanidine (DTG). Although there is evidence that 3-PPP produces some of its in vivo effects via a dopaminergic and not a direct σ mechanism (5), both agents bind at σ sites with high affinity (IC_{50} values ca 25–75 nM), and [^{3}H](+)3-PPP and [^{3}H]DTG have been used as radioligands (6,11). Evidence suggests that σ sites may play a role in mental disorders and, in particular, in schizophrenia (9), and there is a need for new σ-selective agents to further explore this possibility. Because of the putative role of dopamine in various mental disorders, it is particularly important to have available σ agents that lack dopaminergic properties. Indeed, it has been speculated that certain classical neuroleptic agents might act via both a dopamine and σ mechanism (9). The purpose of this preliminary investigation was to identify a new σ ligand with minimal affinity for PCP and dopamine sites. In the course of our work, we have now identified such an agent: R(−)-N-(3-phenyl-n-propyl)-1-phenyl-2-amino-propane hydrochloride [R(−)PPAP)].

METHOD

Radioligand Binding Studies

Competition studies for [^{3}H]haloperidol-labeled σ sites were performed by incubating various concentrations (at least 9) of unlabeled drug with 1.0 nM [^{3}H]haloperidol in the presence of 25 nM spiperone, to preclude binding at dopamine sites, and 3 mg of either rat (Sprague-Dawley) or guinea pig cerebellar homogenate in 2.5 ml of 50 mM Tris HCl buffer (pH 7.7 at 25°C) at room temperature for 90 min. Nonspecific binding was defined in

TABLE 1

RESULTS OF DRUG DISCRIMINATION STUDY WITH (−)PPAP

Agent	Dose (mg/kg)	N*	% Amphetamine-Appropriate Responding (±SEM)†	Response Rate (Resp/min ±SEM)
(+)Amphetamine	1.0	6/6	97 (±1)	16.6 (±2.1)
0.9% Saline (1 ml/kg)		6/6	13 (±4)	15.3 (±1.6)
(−)PPAP	1.0	3/3	8 (±8)	19.9 (±6.9)
	3.0	3/3	2 (±1)	9.3 (±4.1)
	5.0	3/3	13 (±5)	14.2 (±2.8)
	7.0	3/3	6 (±4)	6.9 (±1.5)
	10.0	3/3	3 (±2)	10.9 (±4.0)
	13.0	2/3	15 (±15)	9.4 (±5.4)
	15.0	0/3	—‡	
(−)PPAP +	3.0	4/5	100	19.0 (±4.7)
Amphetamine§	5.0	5/6	100	10.2 (±3.2)
	8.0	2/4	96 (±4)	6.8 (±1.8)
	10.0	2/6	—‡	

*Number of animals making at least 5 total responses during the 2.5-min extinction session/number of animals receiving drug.

†Percent of total responses made on the amphetamine-appropriate lever during the 2.5-min extinction session.

‡Disruption of behavior; majority of animals made fewer than a total of 5 responses.

§Doses of (−)PPAP were administered 5 min prior to administration of the training dose (1 mg/kg) of (+)amphetamine sulfate.

the presence of 10 μM DTG. Incubations were terminated by rapid filtration under vacuum onto glass fiber filters and, after washing with 50 mM Tris HCl buffer, radioactivity was measured using liquid scintillation spectrometry. Competition for [^{3}H]TCP-labeled PCP sites was performed in a similar manner using the procedure of Largent et al. (7) and 3 mg of rat cerebral cortical homogenate. D1 and D2 dopamine binding assays employed [^{3}H]SCH 23390 and [^{3}H]spiperone, respectively, as previously reported (1). All assays were performed at least in triplicate.

Drug Discrimination Studies

Drug discrimination studies were conducted using six male Sprague-Dawley rats (ca. 250–300 g) trained to discriminate 1 mg/kg of S(+)-amphetamine sulfate from saline using a variable interval 15-sec schedule of reinforcement for milk reward. Testing was conducted using standard 2-lever operant chambers (Coulbourn model E10-10). Solutions of all drugs were made fresh daily in 0.9% saline and were injected via the intraperitoneal route 15 min prior to testing. Test sessions were of 2.5-min duration and were conducted under extinction conditions. When R(−)PPAP was administered in combination with S(+)amphetamine, it was administered 5 min prior to the training drug. The training and testing procedures employed are identical to those that we have recently reported; see (3) for details.

The (−)PPAP used in the present study was synthesized in our laboratories by catalytic hydrogenation of hydrocinnamaldehyde and R(−)1-phenyl-2-aminopropane over a 5% Pt/C catalyst; treatment of the crude product with methanolic 10% HCl, and repeated recrystallization of the hydrochloride salt from a mixture of methanol and methyl ethyl ketone afforded a homogeneous product (m.p. 215–217°C). Spectral (infrared and proton NMR) data are consistent with the assigned structure. Elemental analysis was performed by Atlantic Microlab (Norcross, GA) and the values determined for carbon, hydrogen and nitrogen are within 0.4% of theory.

RESULTS AND DISCUSSION

(−)PPAP binds at [^{3}H]haloperidol-labeled rat σ sites with an affinity (IC_{50} = 24 ± 8 nM) greater than that of NANM (IC_{50} = 400 nM). A similar affinity was obtained for the binding of R(−)PPAP at [^{3}H]-haloperidol-labeled guinea pig σ sites (IC_{50} = 23 ± 5 nM). More importantly, unlike NANM, R(−)PPAP does not bind at [^{3}H]TCP-labeled PCP sites (IC_{50} > 75,000 nM). Furthermore, unlike haloperidol which binds at D1 and D2 dopamine receptors (IC_{50} = 60 and 2 nM, respectively), R(−)PPAP displays a very low affinity for these receptors (IC_{50} > 10,000 and > 5,000 nM, respectively). These data suggest that there should be virtually no direct effect of (−)PPAP at PCP sites or at D1 and D2 dopamine receptors in vivo.

Because (−)PPAP possesses a structural similarity to amphetamine (and, to a lesser degree, to 3-PPP), the possibility exists that, like amphetamine, it may be an indirect acting dopamine agonist. In order to test this possibility, tests of stimulus generalization were conducted using rats trained to discriminate 1 mg/kg of S(+)-amphetamine sulfate (ED_{50} dose = 0.4 mg/kg) from saline using standard two-lever operant chambers and a variable-interval 15-sec schedule of reinforcement for food reward. Using a 15-min presession injection interval, groups of rats were administered intraperitoneal doses of (−)PPAP ranging from 1 to 15 mg/kg (Table 1). Under these conditions, amphetamine-stimulus generalization typically occurs both with direct-acting and indirect-acting dopamine agonists (10). However, stimulus generalization was not observed with (−)PPAP; doses of up to 13 mg/kg produced < 16% amphetamine-appropriate responding and 15 mg/kg resulted in disruption of behavior. These results suggest that (−)PPAP is not an indirect-acting dopaminergic amphetamine-

like agonist. In a separate series of studies, attempts were made to antagonize the amphetamine-stimulus; here also, (−)PPAP was without effect (Table 1).

Taken together, the results of the present study suggest that (−)-PPAP is a novel σ-selective ligand that binds at σ sites with an affinity comparable to that of the standard agents (+)3-PPP and DTG. Because (−)PPAP lacks appreciable affinity for dopamine receptors, and does not produce amphetamine-like stimulus effects, it may be of value in the subsequent characterization of σ pharmacology.

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