Radiosynthesis, Cerebral Distribution, and Binding of [^{125}I]-1-(*p*-Iodophenyl)-3-(1-adamantyl)guanidine, a Ligand for σ Binding Sites

Alane S. Kimes,*,† Alan A. Wilson,‡,‖ Ursula Scheffel,‡ Bruce G. Campbell,† and Edythe D. London†,‡,§

Addiction Research Center, National Institute on Drug Abuse, P.O. Box 5180, Baltimore, Maryland 21224, and Division of Nuclear Medicine, Department of Radiology, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21287

Received April 2, 1992

An analog of 1,3-di-*o*-tolylguanidine (DTG), [^{125}I]-labeled 1-(*p*-iodophenyl)-3-(1-adamantyl)-guanidine (PIPAG), was synthesized as a potential ligand for cerebral σ binding sites. Data from in vitro binding experiments and in vivo experiments on brain distribution suggested that PIPAG binds to σ binding sites with high affinity (K_d in low nanomolar range) as determined by Scatchard analysis and relative potencies of σ-specific drugs. Haloperidol had the highest potency to inhibit [^{125}I]PIPAG binding. It was followed by DTG, BMY 14802, and (+)-*N*-allylnormetazocine. Compounds with high affinities for dopamine receptors (but low affinity for σ binding sites), for opioid receptors, for nicotinic acetylcholine receptors, and for phencyclidine receptors were ineffective inhibitors of [^{125}I]PIPAG binding.

Introduction

Recent interest in σ binding sites largely reflects the putative role of these sites in mediating antipsychotic effects of typical and atypical neuroleptic drugs.[1,2] In addition, σ binding sites are localized to tissues of the immune and endrocrine systems,[3,4] and they have been implicated in movement disorders.[5,6] Several recent reviews have focused on the biological functions of σ binding sites.[7-12] The lack of selective, high affinity radioligands has hampered investigations into the properties of σ binding sites and their biochemical role in normal and diseased states. For example, the prototypical σ ligand *N*-allylnormetazocine (NANM, SKF 10,047) has low affinity for the σ receptor and even lower, but substantial, affinity for the phencyclidine (PCP) receptor,[13] whereas haloperidol, the most potent σ ligand yet identified, has equal affinity for σ and dopamine D_2 receptors.[14,15]

A new class of compounds, of which 1,3-di-*o*-tolylguanidine (DTG) is the lead structure, possess high affinity for σ binding sites.[16] These 1,3-disubstituted guanidines are amenable to considerable structural modification while still retaining biological efficacy.[17]

The aim of the present work was to develop an [^{125}I]-labeled radioligand that could serve as a pharmacological tool to study σ binding sites, and which, in its [^{123}I]-labeled form, could serve as a radioligand to image σ binding sites in human brain using single photon emission computed tomography (SPECT). We report here the radiosynthesis and preliminary biological and pharmacological evaluation of a novel [^{125}I]-labeled ligand based upon the DTG template. This compound, 1-(*p*-iodophenyl)-3-(1-adamantyl)guanidine (PIPAG), displays high affinity for the σ receptor.

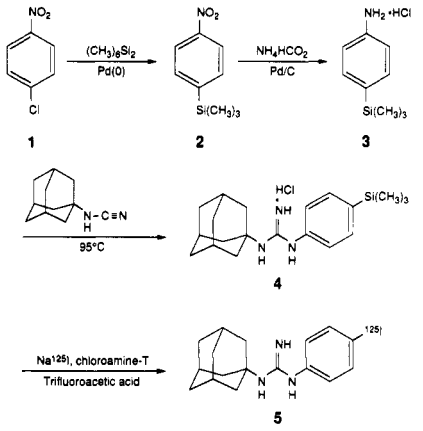
Results and Discussion

Chemistry. The synthetic route followed to prepare [^{125}I]PIPAG is depicted in Scheme I. Attempts to prepare [^{125}I]PIPAG by exchange labeling with 1-(4-bromophenyl)-3-(1-adamantyl)guanidine, using a variety of procedures,[18,19] were unsuccessful, resulting in low and highly variable radiochemical yields. Electrophilic radioiododes-

† National Institute on Drug Abuse.
‡ The Johns Hopkins Medical Institutions.
§ Department of Pharmacology and Experimental Therapeutics, School of Medicine, University of Maryland, Baltimore, MD 21201.
‖ Present address: PET Imaging Facility, Clarke Institute of Psychiatry, 250 College Street, Toronto, Ontario, Canada M5T 1R8.

(1) Snyder, S. H.; Largent, B. L. Receptor mechanisms in antipsychotic drug action: Focus on *sigma* receptors. *J. Neuropsych.* **1989**, *1*, 7-15.
(2) Largent, B. L.; Wikström, H.; Snowman, A. M.; Snyder, S. H. Novel antipsychotic drugs share high affinity for σ receptors. *Eur. J. Pharmacol.* **1988**, *155*, 345-347.
(3) Wolfe, S. A., Jr.; Culp, S. G.; De Souza, E. B. σ-receptors in endocrine organs: Identification, characterization, and autoradiographic localization in rat pituitary, adrenal, testis, and ovary. *Endocrinology* **1989**, *124*, 1160-1172.
(4) Su, T.-P.; London, E. D.; Jaffe, J. H. Steroid binding at σ receptors suggests a link between endocrine, nervous and immune systems. *Science* **1988**, *240*, 219-221.
(5) Matsumoto, R. R.; Bowen, W. D.; Walker, J. M. Age-related differences in the sensitivity of rats to a selective *sigma* ligand. *Brain Res.* **1989**, *504*, 145-148.
(6) Walker, J. M.; Matsumoto, R. R.; Bowen, W. D.; Gans, D. L.; Jones, K. D.; Walker, F. O. Evidence for a role of haloperidol-sensitive σ-"opiate" receptors in the motor effects of antipsychotic drugs. *Neurology* **1988**, *38*, 961-965.
(7) Junien, J. L.; Leonard, B. E. Drugs acting on σ and phencyclidine receptors: A review of their nature, function, and possible therapeutic importance. *Clin. Neuropharmacol.* **1989**, *12*, 353-374.
(8) Deutsch, S. I.; Weizman, A.; Goldman, M. E.; Morihisa, J. M. The *sigma* receptor: A novel site implicated in psychosis and antipsychotic drug efficacy. *Clin. Neuropharmacol.* **1988**, *11*, 105-119.
(9) Chavkin, C. The *sigma* enigma: Biochemical and functional correlates emerge for the haloperidol-sensitive *sigma* binding site. *Trends Pharmacol. Sci.* **1990**, *11*, 213-215.
(10) Sonders, M. S.; Keana, J. F. W.; Weber, E. Phencyclidine and psychotomimetic *sigma* opiates: Recent insights into their biochemical and physiological sites of action. *Trends Neurosci.* **1988**, *11*, 37-40.
(11) Walker, J. M.; Bowen, W. D.; Walker, F. O.; Matsumoto, R. R.; de Costa, B.; Rice, K. C. *Sigma* receptors: Biology and function. *Pharmacol. Rev.* **1990**, *42*, 355-402.
(12) Su, T.-P. σ receptors: Putative links between nervous, endocrine and immune systems. *Eur. J. Biochem.* **1991**, *200*, 633-642.
(13) Largent, B. L.; Gundlach, A. L.; Snyder, S. H. Pharmacological and autoradiographic discrimination of *sigma* and phencyclidine receptor binding sites in brain with (+)-[^{3}H]SKF 10,047, (+)-[^{3}H]-3-[3-hydroxyphenyl]-N-(1-propyl)piperidine and [^{3}H]-1-[1-(2-thienyl)cyclohexyl]piperidine. *J. Pharmacol. Exp. Ther.* **1986**, *238*, 739-748.
(14) Bowen, W. D.; Moses, E. L.; Tolentino, P. J.; Walker, J. M. Metabolites of haloperidol display preferential activity at σ receptors compared to dopamine D-2 receptors. *Eur. J. Pharmacol.* **1990**, *177*, 111-118.
(15) Kuhar, M. J.; Murrin, L. C.; Malouf, A. T.; Klemm, N. Dopamine receptor binding *in vivo*: The feasibility of autoradiographic studies. *Life Sci.* **1978**, *22*, 203-210.
(16) Weber, E.; Sonders, M.; Quarum, M.; McLean, S.; Pou, S.; Keana, J. F. W. 1,3-Di(2-[5-^{3}H]tolyl)guanidine: A selective ligand that labels σ-type receptors for psychotomimetic opiates and antipsychotic drugs. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 8784-8788.
(17) Scherz, M. W.; Fialeix, M.; Fischer, J. B.; Reddy, N. L.; Server, A. C.; Sonders, M. S.; Tester, B. C.; Weber, E.; Wong, S. T.; Keana, J. F. W. Synthesis and structure-activity relationships of N,N'-di-o-tolylguanidine analogues, high-affinity ligands for the haloperidol-sensitive σ receptor. *J. Med. Chem.* **1990**, *33*, 2421-2429.

Scheme I. Synthesis of Precursor to and Radiosynthesis of [^{125}I]PIPAG



silylation has proven to be a useful method for the introduction of radioiodine into complex molecules at high specific activity;[20-23] therefore, the trimethylsilylguanidine (4) was targeted as a suitable precursor for the radiosynthesis of [^{125}I]PIPAG.

The difficulties associated with introducing a trimethylsilyl group into an aromatic ring in the presence of a nitro group[24] were overcome using the Pd(0)-catalyzed reaction of 1 with hexamethyldisilane. This reaction was developed by Matsumoto.[25] Reduction of the nitro group[26] of 2 and acidification gave trimethylsilylaniline (3) as the hydrochloride salt in nearly 100% yield. 1-Aryl-3-(1-adamantyl)guanidines are conveniently prepared by the addition of substituted aniline hydrochlorides to 1-adamantylcyanamide.[27] However, when 3 was reacted with 1-adamantylcyanamide in a variety of solvents (DMF, HMPA, ethanol, acetonitrile, glyme), very little of the desired product, 4, was obtained.

Aniline and 1-phenyl-3-(1-adamantyl)guanidine were the major products of the attempted addition of 3 with cyanamide, indicating that desilylation was competing successfully with the desired addition reaction. The aryl-silicon bond has a propensity for acid-induced cleavage that is exacerbated by the electron-donating amino group[26] of 3. Heating a finely ground mixture of 3 and the cyanamide in the absence of any solvent[28] provided a reasonable (39%) yield of 4, the desired precursor for radioiodination. This semimelt reaction increased the effective concentration of both reagents compared to solution reactions, thus facilitating the bimolecular addition reaction of aniline to cyanamide at the expense of the unimolecular desilylation reaction.

Electrophilic radioiododesilylation of the (trimethylsilyl)guanidine 4 to produce [^{125}I]PIPAG proceeded smoothly as outlined in Scheme I. At ambient temperature, sodium [^{125}I]iodide was oxidized by chloramine-T with trifluoracetic acid as the solvent[23] and was reacted with 4. More than 90% of the radioactivity was incorporated into the product after 15 min, as measured by HPLC. Unreacted precursor 4 and other chemical and radiochemical impurities were effectively removed by purification of the reaction mixture by semipreparative reverse-phase HPLC. Upon formulation, [^{125}I]PIPAG was obtained, with 55–72% isolated radiochemical yield, in a synthetic time of 40–45 min. Specific activities ranged from 200 to 1200 mCi/μmol, and radiochemical purity was >98%, as determined by analytical HPLC. The characteristics of this reaction (short reaction times, mild reaction conditions, high radiochemical yields, specific activities, and purities) demonstrated that preparation of [^{123}I]-PIPAG is feasible.

In Vitro Binding Studies. [^{125}I]PIPAG bound to specific sites in membranes from guinea pig brain. A typical experiment in which 0.07 nM [^{125}I]PIPAG was incubated with membranes gave 13000 cpm of total binding and 2000 cpm of nonspecific binding as defined by inhibition with 50 μM haloperidol. Binding was linearly proportional to the amount of membrane protein (0.01–0.3 mg). Maximum specific binding occurred within 120 min at 25 °C, and this binding was reversible upon addition of unlabeled PIPAG. The pH optimum for specific binding was 7.8. Binding to the filters was 300–400 cpm in the absence of tissue.

Saturation Studies. Scatchard analysis of data obtained from two experiments in which [^{125}I]PIPAG binding sites were saturated by diluting the radioligand with unlabeled PIPAG (0.5–125 nM) revealed a single recognition site for [^{125}I]PIPAG (Figure 1). Calculated values for K_d from these experiments were 8.7 and 11.2 nM, and values for B_{max} were 33.8 and 42.5 pmol/mg of protein. Hill coefficients were 0.97 and 1.01. The analysis of data obtained from three experiments using concentrations of [^{125}I]PIPAG ranging from 0.044 to 10 nM also revealed a single recognition site for [^{125}I]PIPAG. The calculated values of K_d and B_{max} were 3.8 ± 0.8 nM and 10.3 ± 3.0 pmol/mg of protein, respectively. The Hill coefficient of binding was 1.01 ± 0.003. The fact that the values for K_d and B_{max} in these latter studies were slightly lower (albeit similar) may reflect the inherent difficulties associated with the accurate determination of mass for radioligands of very high specific activity. In addition, concentrations of [^{125}I]PIPAG in these experiments were insufficient to exceed the inflection point in the plot of radioactivity

(18) Mertens, J.; Callaerts, A.; Vanryckeghem, W. New realistic approach of the kinetics in fast labelling procedures. *J. Labelled Compd. Radiopharm.* **1987**, *24*, 612–617.

(19) Moerlein, S. M.; Hwang, D.-R.; Welch, M. J. No-carrier-added radiobromination via cuprous chloride-assisted nucleophilic aromatic bromodeiodination. *Int. J. Rad. Appl. Instrum. [A]* **1988**, *39*, 369–372.

(20) Ransom, R. W.; Eng, W.-S.; Burns, H. D.; Gibson, R. E.; Solomon, H. F. (+)-3-[^{123}I]Iodo-MK-801: Synthesis and characterization of binding to the N-methyl-D-aspartate receptor complex. *Life Sci.* **1990**, *46*, 1103–1110.

(21) Wilbur, D. S.; Anderson, K. W.; Stone, W. E.; O'Brien, H. A., Jr. Radiohalogenation of non activated aromatic compounds via aryltrimethylsilyl intermediates. *J. Labelled Compd. Radiopharmacol.* **1982**, *19*, 1171–1188.

(22) Wilson, A. A.; Dannals, R. F.; Ravert, H. T.; Burns, H. D.; Wagner, H. N., Jr. I-125 and I-123 labelled iodobenzyl bromide, a useful alkylating agent for radiolabelling biologically important molecules. *J. Labelled Compd. Radiopharmacol.* **1986**, *23*, 83–93.

(23) Wilson, A. A.; Dannals, R. F.; Ravert, H. T.; Frost, J. J.; Wagner, H. N., Jr. Synthesis and biological evaluation of [^{125}I]- and [^{123}I]-4-iododexetimide, a potent muscarinic cholinergic receptor antagonist. *J. Med. Chem.* **1989**, *32*, 1057–1062.

(24) Häbich, D.; Effenberger, F. Preparation of aryl- and heteroaryltrimethylsilanes. *Synthesis* **1979**, 841–876.

(25) Matsumoto, H.; Nagashima, S.; Yoshihiro, K.; Nagai, Y. Silicon–carbon bond formation by the reaction of disilanes with halobenzenes in the presence of tetrakis(triphenylphosphine)palladium(0). *J. Organomet. Chem.* **1975**, *85*, C1–C3.

(26) Benkeser, R.; Bromfield, P. Aminotrialkylsilanes. *J. Am. Chem. Soc.* **1952**, *74*, 253–254.

(27) Safir, S. R.; Kushner, S.; Brancone, L. M.; Subbarow, Y. Experimental chemotherapy of trypanosomiasis. II. The preparation of compounds related to *p*-phenylenediguanidine. *J. Org. Chem.* **1948**, *13*, 924–932.

(28) Geluk, H. W.; Schut, J.; Schlatmann, J. L. M. A. Synthesis and antiviral properties of 1-adamantylguanidine. A modified method for preparing *t*-alkylguanidines. *J. Med. Chem.* **1969**, *12*, 712–715.

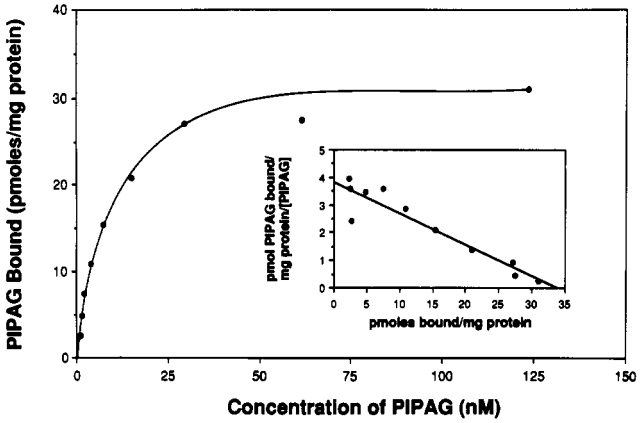


Figure 1. Equilibrium binding of [125I]PIPAG to guinea pig membranes. Saturation isotherm: membranes (80 μg of protein) were incubated for 120 min at 4 °C with 0.5 nM of [125I]PIPAG and increasing concentrations of unlabeled PIPAG were added to achieve final concentrations of 0.5 to 125 nM. Nonspecific binding was measured in the presence of 50 μM haloperidol. Inset: Scatchard plot. The data are from a selected experiment. All assays were performed in triplicate. K_d and B_{max} were determined by computer (LIGAND programs).

bound as a function of the free ligand concentration.[29] Plots of bound ligand versus free ligand concentration for experiments in which [125I]PIPAG was diluted with unlabeled PIPAG confirmed that the highest concentration of PIPAG exceeded the inflection point, suggesting that the estimate of B_{max} from these experiments is more reliable.

In previous studies, analysis of the experimental data distinguished two binding sites for some σ ligands, providing evidence for heterogeneity of σ binding sites.[30-32] Inhibition studies suggested that both sites were σ binding sites. However, if [125I]PIPAG binds to more than one site, it binds to them with equal affinity and does not distinguish between them. Values for B_{max} obtained in these studies generally are higher than those obtained using other ligands for σ receptor binding. The reason for this discrepancy is not clear. It may reflect binding of [125I]-PIPAG to non-σ sites, although the results of competition studies indicate selectivity of PIPAG for σ sites.

Inhibition Studies. The rank order of potency of inhibitors of [125I]PIPAG binding is similar to their inhibition of binding by other σ ligands (Table I). Haloperidol was the most potent, followed by DTG and BMY 14802. As observed with other σ ligands, there was stereoselectivity for the (+)- and (−)-enantiomers of NANM, with (+)-NANM being a more potent inhibitor of [125I]PIPAG binding than (−)-NANM. Compounds that typically bind to other receptors (phencyclidine receptors, dopamine receptors, opioid receptors, and nicotinic acetylcholine receptors) and not to σ binding sites were ineffective (up to concentrations of 10 μM) at inhibiting the binding of [125I]PIPAG. There was a tendency for

Table I. IC_{50} Values of Various Drugs in Competition with Radiolabeled σ Ligands

	IC_{50} (nM)			
inhibitor	[125I]PIPAG[a]	[3H]DTG[b]	[3H]-3-PPP[b]	[3H]NANM[b]
haloperidol	8 ± 3	5[16]	17[16]	4[35]
DTG	18 ± 5	28[16]	53[16]	15[11]
BMY 14802	71 ± 33	120[46]		
(+)-NANM	735 ± 8	625[16]	93[16]	48[35]
rimcazole	780 ± 277	1400[46]		450[49]
PCP	868 ± 328	1050[16]	1000[16]	560[50]
TCP	2060 ± 1290			1100[46]
(−)-NANM	5000 ± 181	4000[16]	2850[34]	1800[35]
dizocilpine	>10000			>10000[46]
naloxone	>10000			>100000[35]
apomorphine	>10000	>10000[16]		>31000[35]
sulpiride	>10000			>100000[46]
l-nicotine	>10000			

[a] Binding of 0.07 nM [125I]PIPAG was assayed in crude preparations of whole guinea pig brains. IC_{50} values (concentration inhibiting 50% of specific binding) were determined on a log-logit scale. Data from studies of [125I]PIPAG binding are shown as means ± SEM of at least three assays performed in duplicate. [b] Published data; references as noted.

shallow or biphasic inhibition curves for the inhibition of binding by haloperidol, (+)-NANM, and DTG (Hill coefficients: 0.59 ± 0.12, 0.68 ± 0.17, and 0.59 ± 0.06, respectively), suggesting that these drugs inhibit the binding of [125I]PIPAG to more than one receptor.

The K_d for PIPAG reported herein is similar to the IC_{50} of 2.8 nM against [3H]DTG reported for this compound previously.[33] The pattern of ligand selectivity and the relative potencies, as judged by the IC_{50} values of relevant drugs in inhibiting [125I]PIPAG binding (Table I), is similar to that reported for σ binding sites using [3H]DTG[16] (also shown in the table). Although the rank order of these drugs is also similar to that for the inhibition of [3H]-3-PPP and [3H]NANM binding, (+)- and (−)-NANM are considerably less potent in inhibiting [125I]PIPAG binding than the binding of [3H]-3-PPP and [3H]NANM.[34,35] In contrast, (+)-NANM and (−)-NANM inhibition of [3H]-DTG binding is about equipotent to the inhibition of [125I]-PIPAG.[16] It is not surprising that PIPAG would have a similar pharmacological profile to DTG, given the structural similarity of the two drugs.

Autoradiography. The autoradiographic distribution of [125I]PIPAG binding sites in guinea pig brain is shown in Figure 2. Nonspecific binding was uniformly low throughout the brain. Specific [125I]PIPAG binding to σ binding sites was found throughout the brain, with the highest densities in the cerebellum and pons. Specific binding increased in a rostral to caudal direction in the guinea pig brain, with very little specific binding in the caudate putamen, the frontal cortex, or thalamic areas. In contrast, the entorhinal cortex, preoptic nucleus, pontine nucleus, and paralemniscal nucleus, as well as the cerebellum, contained high densities of binding sites. Medullary cranial nerve nuclei, such as the caudal spinal trigeminal nucleus and the hypoglossal nucleus, showed

(29) Klotz, I. M. Numbers of receptor sites from Scatchard graphs: Facts and fantasies. *Science* **1982**, *217*, 1247–1249.
(30) Bowen, W. D.; Hellewell, S. B.; McGarry, K. A. Evidence for a multi-site model of the rat brain σ receptor. *Eur. J. Pharmacol.* **1989**, *163*, 309–318.
(31) Wu, X.-Z.; Bell, J. A.; Spivak, C. E.; London, E. D.; Su, T.-P. Electrophysiological and binding studies on intact NCB-20 cells suggest presence of a low affinity *sigma* receptor. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 351–359.
(32) Quirion, R.; Bowen, W. D.; Itzhak, Y.; Junien, J. L.; Musacchio, J. M.; Rothman, R. B.; Su, T.-P.; Tam, S. W.; Taylor, D. P. A proposal for the classification of *sigma* binding sites. *Trends Pharmacol. Sci.* **1992**, *15*, 85–86.
(33) Wilson, A. A.; Dannals, R. F.; Ravert, H. T.; Sonders, M. S.; Weber, E.; Wagner, H. N., Jr. Radiosynthesis of σ receptor ligands for positron emission tomography: ^{11}C- and ^{18}F-labeled guanidines. *J. Med. Chem.* **1991**, *34*, 1867–1870.
(34) Largent, B. L.; Gundlach, A. L.; Snyder, S. H. Psychotomimetic opiate receptors labeled and visualized with (+)-[3H]-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 4983–4987.
(35) Tam, S. W.; Cook, L. σ opiates and certain antipsychotic drugs mutually inhibit (+)-[3H]SKF 10,047 and [3H]haloperidol binding in guinea pig brain membranes. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 5618–5621.

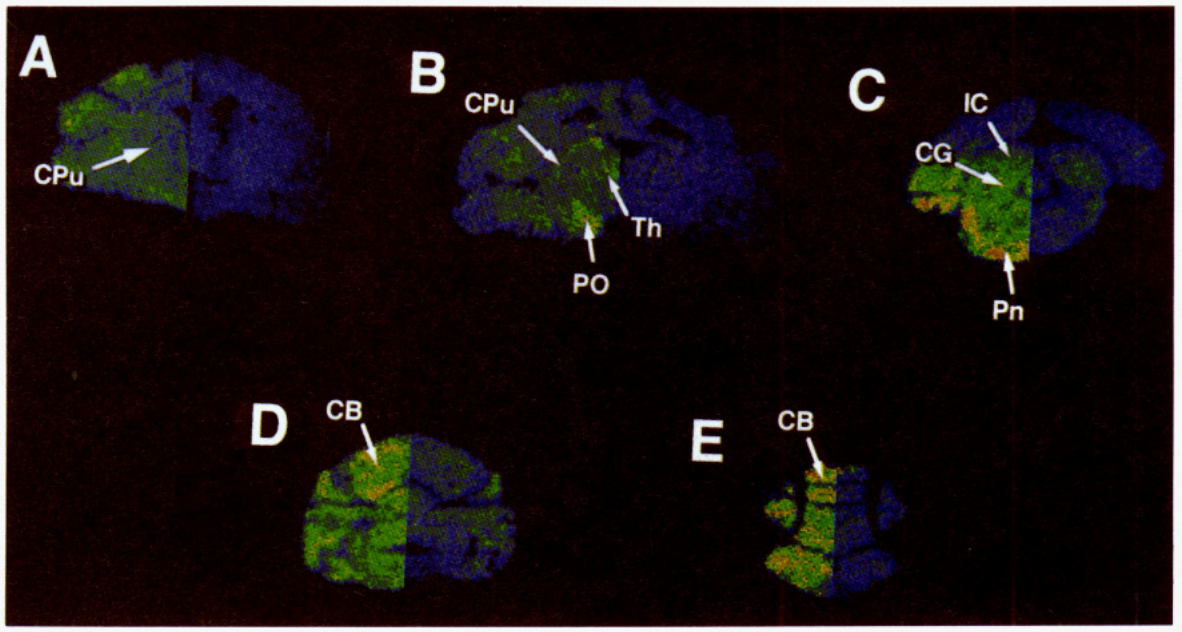


Figure 2. Autoradiograms showing the distribution of binding sites in coronal sections of guinea pig brain labeled by [^{125}I]PIPAG in the absence (left hemispheres) and presence (right hemispheres) of 50 μM haloperidol. (A) A section midway through the caudate putamen. (B) A section through the anterior thalamus. (C) A section through the midbrain at the level of the inferior colliculus and central gray. (D) A section through the cerebellum and pons. (E) A section through the caudal cerebellum and medulla. Abbreviations: CPu, caudate putamen; PO, preoptic nucleus, Th, anterior thalamus; IC, inferior colliculus; Pn, pontine nucleus; CG, central gray; CB, cerebellum.

high densities of [^{125}I]PIPAG binding sites. The distribution of [^{125}I]PIPAG binding was remarkably similar to that of [^{3}H]-3-PPP binding to guinea pig brain slices, as shown by Largent et al.[13,34,36] Binding of [^{125}I]PIPAG in vitro did not correlate well with either the in vitro binding to guinea pig brain slices of [^{3}H]NANM,[13] which appeared to bind to both σ and PCP binding sites, or that of [^{3}H]-haloperidol[37] to rat brain slices (in the presence of spiroperidol to block D_2 dopamine receptors). [^{3}H]-Haloperidol labeled more sites in the caudate putamen and hippocampus and fewer sites in the pons medulla than either [^{125}I]PIPAG or [^{3}H]-3-PPP. The concentration of spiroperidol used may have been insufficient to inhibit binding of [^{3}H]haloperidol to dopamine receptors, especially in the dopamine receptor rich caudate putamen.[38] Furthermore, the distribution of σ binding sites (labeled by [^{3}H]haloperidol) in rat brain may be somewhat different from those in guinea pig brain.

Biodistribution Studies. The distribution of radioactivity in mouse brain at various time intervals after intravenous injection of [^{125}I]PIPAG (2.0 μCi, 0.13 μg/kg) was determined by dissection and counting of dissected brain regions. γ counting of the excised tissue revealed a regional distribution (specific binding in cerebellum, 0.64% injected dose; striatum, 0.58%; thalamus, 0.54%; cortex, 0.53%; and hippocampus, 0.46%) consistent with the observations of Weissman et al.,[39] who measured [^{3}H]-haloperidol uptake in mouse brain, in the presence of spiroperidol to block binding to D_2 receptors. These data suggest that the regional distribution of σ sites in mouse brain may be less heterogeneous than the distribution of σ sites in guinea pig brain. Furthermore, although autoradiographic studies in guinea pigs and rats have identified discrete brain regions of high σ receptor concentrations,[16,36,40] the resolution of those studies was much greater than that attainable by dissection techniques. As uptake of [^{125}I]PIPAG was highest in the cerebellum, this region was selected to demonstrate the time course of brain radioactivity in the presence and absence of haloperidol (Figure 3).

Within 15 min after iv injection of [^{125}I]PIPAG, 0.6%/g of the injected dose was found in the cerebellum, and this modest level of activity was maintained over 5 h (Figure 3). Preinjection of haloperidol (1 mg/kg) resulted in a time-dependent decrease in brain radioactivity levels (Figure 3) with 24, 39, and 79% reductions after 30, 60, and 300 min, respectively. This blocking effect of haloperidol occurred in a dose-dependent manner with an ED_{50} of approximately 0.1 mg/kg at 300 min after the injection of [^{125}I]PIPAG. Uptake of radioactivity, measured at 300 min after the injection, was also inhibited by preinjection of unlabeled PIPAG in a dose-dependent manner, with an ED_{50} of about 1.0 mg/kg.

A preinjection of haloperidol (1 mg/kg) significantly reduced the uptake of the radioligand by 70–80% in all mouse brain regions examined, whereas a preinjection of spiroperidol (1 mg/kg) had no significant effect on

(36) Gundlach, A. L.; Largent, B. L.; Snyder, S. H. Autoradiographic localization of *sigma* receptor binding sites in guinea pig and rat central nervous system with (+)-[^{3}H]-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine. *J. Neurosci.* **1986**, *6*, 1757–1770.

(37) Contreras, P. C.; Quirion, R.; Gehlert, D. R.; Contreras, M. L.; O'Donohue, T. L. Autoradiographic distribution of non-dopaminergic binding sites labeled by [^{3}H]haloperidol in rat brain. *Neurosci. Lett.* **1987**, *75*, 133–140.

(38) Vu, T. H.; Weissman, A. D.; London, E. D. Pharmacological characteristics and distributions of σ- and phencyclidine receptors in the animal kingdom. *J. Neurochem.* **1990**, *54*, 598–604.

(39) Weissman, A. D.; Broussolle, E. P.; London, E. D. *In vivo* binding of [^{3}H]*d*-N-allylnormetazocine and [^{3}H]haloperidol to *sigma* receptors in the mouse brain. *J. Chem. Neuroanat.* **1990**, *3*, 347–354.

(40) Graybiel, A. M.; Besson, M.-J.; Weber, E. Neuroleptic-sensitive binding sites in the nigrostriatal system: Evidence for differential distribution of *sigma* sites in the subtantia nigra, pars compacta of the cat. *J. Neurosci.* **1989**, *9*, 326–338.

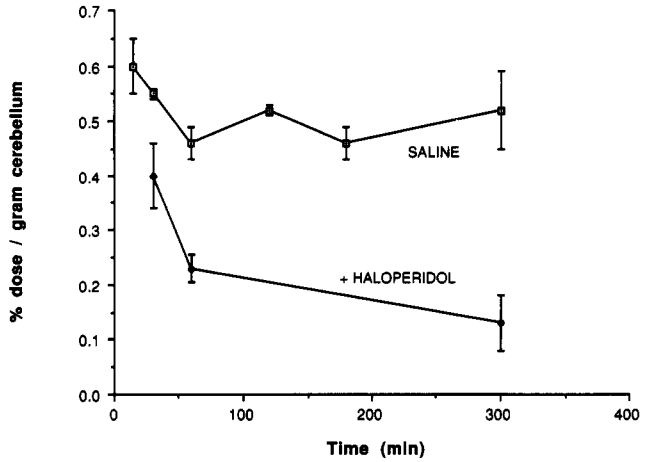


Figure 3. Time course of the uptake of [125I]PIPAG in mouse cerebellum. Haloperidol (1 mg/kg) or saline was administered iv, 5 min before injection of the radioligand. Data are given as means ± SEM for n = 4 mice.

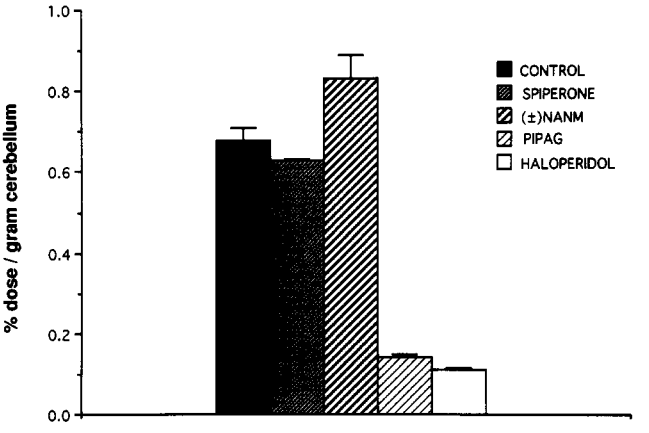


Figure 4. Blocking effect of a preinjection of spiroperidol (1 mg/kg), (±)-NANM (5 mg/kg), unlabeled PIPAG (5 mg/kg), or haloperidol (1 mg/kg) on the uptake of [125I]PIPAG in the cerebellum of mouse brain at 300 min postinjection. Data are given as means ■ SEM for n = 4 mice. Similar findings were obtained in hippocampus, striatum, cortex, and thalamus.

radioactivity levels in any region. Unlabeled PIPAG (5 mg/kg) also reduced radioactivity levels to values comparable to those measured using haloperidol (1 mg/kg) as the blocker. Preadministration of racemic (±)-NANM (5 mg/kg), however, did not block uptake of the [125I]-labeled radioligand. Data for the effect of these drugs on uptake of [125I]PIPAG in the cerebellum are shown in Figure 4.

The following observations suggest that uptake of radioactivity in mouse brain after injection of [125I]PIPAG is associated with binding to σ binding sites: (1) binding exhibits dose-dependent saturation, manifested by preinjection of unlabeled PIPAG or haloperidol; and (2) the potent σ ligand, haloperidol, has a dose-dependent blocking effect. The involvement of catecholamine binding sites was investigated using a preinjection of spiroperidol, as haloperidol is equipotent at dopamine D_2 and σ binding sites and has considerable affinity for α_1-adrenergic receptors.[39] The inability of spiroperidol to reduce uptake of radioactivity would appear to rule out dopamine D_2 and α_1-adrenergic receptor involvement.

Preinjection of (±)-NANM (5 mg/kg) failed to inhibit uptake of [125I]PIPAG (Figure 4). This observation probably reflects the low inhibitory potency of either stereoisomer of NANM in the in vitro competition studies with [125I]PIPAG. The affinity of racemic NANM for σ binding sites is over 100-fold less than the affinity of haloperidol for σ binding sites;[13,41] doses of 25 mg/kg of the more potent dextrorotatory stereoisomer are required to produce significant inhibition of [18F]haloperidol uptake in mouse cerebellum.[42]

Conclusion

[125I]PIPAG was synthesized as a potential radioligand for the σ receptor. The pharmacology of the radioligand, as evidenced from inhibition by a series of σ-selective compounds and other potential inhibitors, indicated that the radioligand is selective for σ binding sites. The radiotracer has been labeled with 125I, rendering it suitable for in vitro autoradiographic studies, with relatively short exposure times as compared with tracers labeled with tritium. The potential utility of [123I]PIPAG for SPECT imaging of σ binding sites warrants further evaluation. The fact that only a moderate amount (0.6% of injected dose/g of tissue in mouse brain) penetrates the blood–brain barrier may limit its usefulness as a SPECT ligand for human studies because of radiation dosimetry considerations.

Experimental Section

Purifications and analyses of 125I-containing radioactive mixtures were performed on an HPLC system composed of a Rheodyne 7125 injector, two Waters 510 EF pumps, a UV detector (Waters 481), and an Ortec flow radioactivity detector. The synthetic route followed to prepare [125I]PIPAG is depicted in Scheme I. Peak areas were measured using Hewlett-Packard 3390A recording integrators. Isolated radiochemical yields were determined with a dose calibrator (Capintec CRC-7). Sodium [125I]iodide was obtained from Amersham Corp. (IMS-30). NMR spectra were obtained on an IBM NR/80 using $(CH_3)_4Si$ as an internal standard. Elemental analyses were performed by Atlantic Microlab (Atlanta, GA). New compounds gave satisfactory elemental analyses (C, H, N ±0.4%), except as noted. Melting points were uncorrected.

4-(Trimethylsilyl)-1-nitrobenzene (2). A mixture of 1-chloro-4-nitrobenzene (1) (0.5 g, 3.2 mmol), hexamethyldisilane (2.0 g, 13.5 mmol), and tetrakis(triphenylphosphine)palladium(0) (45 mg, 38.9 μmol; Aldrich Chemical Co., Inc., Milwaukee, WI) in xylene (1.5 mL) was sealed under argon and stirred at 150–160 °C for 3 h. The mixture was cooled; 40 mL of petroleum ether (bp 30–60 °C) was added, and the mixture was filtered. Evaporation of the filtrate left a dark red oil which solidified upon drying in vacuo. The product was isolated by column chromatography (silica) using 10–20% methylene chloride in hexane as eluent (0.405 g, 65%): mp 34–36 °C (lit.[43] mp 37 °C). ^{1}H NMR ($CDCl_3$) δ 0.32 (s, 9 H), 7.6–8.2 (q, 4 H).

4-(Trimethylsilyl)aniline Hydrochloride (3). A stirred solution of 4-(trimethylsilyl)-1-nitrobenzene (0.37 g, 1.89 mmol) and ammonium formate (1.0 g, 15.9 mmol) in methanol (10 mL)[44] under argon was treated with 10% palladium on charcoal (20 mg). After 30 min, the mixture was filtered through Celite and evaporated to dryness. The residue was partitioned between ether and water and the ethereal layer washed with water, dried (Na_2SO_4), and filtered. Ethereal hydrochloric acid was added dropwise to precipitate shiny white crystals that were collected by vacuum filtration and dried in vacuo (0.28 g, 73%): mp 101–

(41) Koe, B. K.; Burkhart, C. A.; Lebel, L. A. (+)-[3H]3-(3-hydroxyphenyl)-N-(1-propyl)-piperidine binding to σ receptors in mouse brain *in vivo*. *Eur. J. Pharmacol.* **1989**, *161*, 263–266.

(42) Wong, D. F.; Gibson, R.; London, E. D.; Burns, H. D.; Shaya, E.; Dannals, R. F.; Ravert, H. T.; Wilson, A. A.; Wagner, H. N., Jr. *In vivo* studies of σ receptors with radiolabeled haloperidol. *Soc. Neurosci. Abstr.* **1990**, *16*, 1140 (Abstract).

(43) Deans, F. B.; Eaborn, C. Organosilicon Compounds. Part XIX. Trimethyl-*p*-nitrophenylsilane. *J. Chem. Soc.* **1957**, *10*, 498–499.

(44) Ram, S.; Ehrenkaufer, R. E. Ammonium formate in organic synthesis: A versatile agent in catalytic hydrogen transfer reductions. *Synthesis* **1988**, 91–95.

103 °C. ^{1}H NMR ($CDCl_3$) δ 0.25 (s, 9 H), 7.52 (s, 4 H), 10.0–10.8 (br s, 3 H, exchangeable).

1-Adamantylcyanamide. 1-Adamantylcyanamide was prepared from 1-adamantylamine and cyanogen bromide by known methods: mp 148–149 °C (lit.[18] mp 150–151 °C).

1-(*p*-Iodophenyl)-3-(1-adamantyl)guanidine (PIPAG). A mixture of 1-adamantylcyanamide (100 mg, 0.57 mmol) and 4-iodoaniline hydrochloride (recrystallized from ethanol/acetone, 158 mg, 0.62 mmol) in a Reactivial (Pierce) was heated to 150 °C for 1 h. The mixture was then vortexed and heated further for 45 min and cooled. The reaction mixture was partitioned between aqueous Na_2CO_3 (4 N) and 50:50 ether/dichloromethane. The organic layer was washed with water, dried (Na_2SO_4), and filtered, and the solvents were removed. The residue was recrystallized from 50% aqueous ethanol and the product isolated as an off-white solid by vacuum filtration (0.125 g, 55.5%): mp 204–206 °C (lit.[33] mp 204–206 °C). ^{1}H NMR ($CDCl_3$) δ 1.64, 2.04, 2.12 (3 overlapping br s, 15 H), 3.75–4.25 (br s, 3 H, exchangeable), 6.56–7.87 (q, 4 H).

1-[4-(Trimethylsilyl)phenyl]-3-(1-adamantyl)guanidine (4). A mixture of 1-adamantylcyanamide (60 mg, 0.34 mmol) and 4-(trimethylsilyl)aniline hydrochloride (75 mg, 0.37 mmol) was heated to 95 °C for 30 min in a Reactivial (Pierce). Partial melting occurred. Upon cooling the mixture was partitioned between ether/dichloroethane (1:1) and aqueous hydrochloric acid (0.1 N). The organic layer was washed with water, dried (Na_2SO_4), and filtered, and volatile fluids were removed to leave an oily solid that crystallized upon trituration with ether as a white powder (50.1 mg, 39%). Recrystallization from ether/dichloromethane provided an analytical sample: mp 206–210 °C dec. Anal. ($C_{20}H_{32}N_3SiCl$) C, H; N: calcd, 11.12; found, 11.18.

[^{125}I]-1-(*p*-Iodophenyl)-3-(1-adamantyl)guanidine ([^{125}I]-PIPAG) (5). A freshly prepared solution of 1-(4-trimethylsilylphenyl)-3-(1-adamantyl)guanidine hydrochloride (0.2–0.6 mg) in trifluoracetic acid (50 μL) was treated with aqueous sodium [^{125}I]iodide (Amersham IMS.30, 2–18 μL, 0.2–1.6 mCi) followed by freshly prepared aqueous chloramine-T (2 mg/mL, 4 μL). The mixture was vortexed and left at ambient temperature for 15 min and then quenched with a mixture of ethanol (50 μL), concentrated aqueous ammonium hydroxide (35 μL), saturated sodium thiosulfate (2 μL), and HPLC eluent (100 μL). The product was then isolated by semipreparative HPLC using a Waters Novapak (25 cm × 7.5 mm) with CH_3CN/H_2O 45:55 + 0.1 N ammonium formate as eluent at 5 mL/min (k'_{PIPAG} 6.2). After evaporation of the appropriate fraction to near dryness, the product was most conveniently taken up in 5% aqueous ethanol. For biological studies, this solution was diluted a minimum of 10-fold with saline. The purity and specific activities of the final product were determined by analytical HPLC (Waters Novapak C18 (15 cm × 3.5 mm) with CH_3CN/H_2O 50:50 + 0.1 N ammonium formate, 2 mL/min (k'_{PIPAG} 3.5), as previously described.[23] Anal. ($C_{17}H_{22}N_3I$) H; C: calcd, 51.66; found, 51.34. N: calcd, 10.63; found, 10.46.

In Vitro Studies. The following drugs were used as potential inhibitors of [^{125}I]PIPAG binding: haloperidol (McNeil Pharmaceutical, Spring House, PA); DTG (Aldrich Chemical Co. Inc.); BMY 14802 (a gift from Bristol-Myers Squibb Co., Wallington, CT); rimcazole (a gift from Burroughs Wellcome, Research Triangle Park, NC); (+)- and (−)-NANM (NIDA, Research Triangle, NC); PCP and 1-[1-(2-thienyl)cyclohexyl]piperidine (TCP; Research Technical Branch, NIDA, Research Triangle, NC); dizocilpine (MK 801; a gift from Merck Sharp & Dohme Research Lab, Rahway, NJ); apomorphine, sulpiride, and naloxone (Sigma Chemical Co., St. Louis, MO); and *l*-nicotine (BDH Chemicals, Ltd., Poole, England).

Tissue Preparation. Frozen whole guinea pig brains were purchased from Pel Freeze Biologicals (Rogers, AR). The brains were homogenized in 10 volumes of ice-cold 320 mM sucrose using a Brinkman Polytron. The homogenate was centrifuged at 1000*g* for 20 min at 4 °C. The supernatant fluid was centrifuged at 20000*g* for 20 min at 4 °C. The resulting pellet was resuspended in 10 volumes of 50 mM Tris HCl buffer at pH 7.4, and the final suspension was adjusted to yield a protein concentration of 1 mg/mL as assayed using bovine serum albumin standards.[45] Aliquots of 20 mL were stored at −70 °C until used, with no detectable loss of binding.[16,46]

[^{125}I]PIPAG Binding Assay. Membranes (80 μg of protein) from whole brain were incubated in glass tubes with 50 mM Tris buffer, pH 7.8, and [^{125}I]PIPAG (specific activity, 450 Ci/mmol) in a total volume of 1 mL at 25 °C. For all assays, except those to determine kinetic parameters of binding, the incubation time was 2 h (see below). Except for saturation studies, the concentration of [^{125}I]PIPAG was 0.07 nM.

Reactions were terminated by dilution with 5 mL of ice-cold buffer followed by filtration under vacuum onto Whatman GF/B glass-fiber filters presoaked in 0.1% polyethylenimine. The apparatus used for filtration was a 48-channel tissue harvester (Brandel, Gaithersburg, MD). Filters were rinsed with two aliquots (5 mL each) of ice-cold buffer, and radioactivity on the filters was measured using a γ counter (Pharmacia LKB Biotechnology, Inc. Model 1277 Gammamaster, Gaithersburg, MD). Nonspecific binding was determined in the presence of haloperidol (50 μM).

Saturation Analyses. The assays were conducted as described above, except that the concentration of [^{125}I]PIPAG in the incubation medium was varied. In some assays (hot Scatchard), eight concentrations of radioligand, 0.044–10 nM, were used with no dilution of specific activity. In other assays (cold Scatchard), the concentration of radioligand was varied from 0.5 to 125 nM by the addition of unlabeled PIPAG, which reduced specific activity of the radioligand. Data were analyzed using an iterative least-squares curve fitting computer program (LIGAND).[47]

Competition Studies. Binding assays were conducted as described above, except that, in addition to assays of total (no inhibitor) and nonspecific binding, other incubations contained various concentrations of competing drugs (10 concentrations/drug). The concentration of inhibitor varied with the competing drug as follows: (+)- and (−)-NANM, PCP, and TCP, (5 nM–100 μM); haloperidol (0.2 nM–10 μM); DTG (0.2 nM–10 μM); BMY 14802 (2 pM–10 μM); and rimcazole (2 pM–10 μM). Values of IC_{50} were estimated by log–logit plots, using EBDA software.[48] Reactions were terminated as described above for saturation studies. Nonspecific binding was determined in the presence of haloperidol (50 μM).

Autoradiography. Harley male guinea pigs, 300–350 g (Hazelton Research Products, Denver, PA) were killed by decapitation. Their brains were removed and frozen on dry ice. The distribution of [^{125}I]PIPAG binding was studied using 10-μm-thick coronal slices of guinea pig brain. Serial sections were incubated with [^{125}I]PIPAG (0.07 nM, specific activity 450 Ci/mmol) for 2 h in an ice bath (4 °C) in 50 mM Tris buffer in the absence or presence of 10 μM haloperidol or 10 μM unlabeled PIPAG. Sections were washed twice (5 min each) in cold buffer and then they were washed in distilled water and rapidly dried under cooled air. The sections were apposed to Ultrofilm ^{3}H (Reichert-Jung, Cambridge Instruments GmbH, Nusslock, Germany) with calibrated standards (Amersham) for 6 days. The developed films were digitized using a Loats Image analysis system (Westminster, MD). Calibrated images were transferred to a

(45) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.

(46) Campbell, B. G.; Scherz, M. W.; Keana, J. F. W.; Weber, E. *Sigma* receptors regulate contractions of the guinea pig illeum longitudinal muscle/myenteric plexus preparation elicited by both electrical stimulation and exogenous serotonin. *J. Neurosci.* **1989**, *9*, 3380–3391.

(47) Munson, P. J.; Rodbard, D. Ligand: A versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **1980**, *107*, 220–239.

(48) McPherson, G. A. Analysis of radioligand binding experiments: A collection of computer programs for the IBM PC. *J. Pharmacol. Methods* **1985**, *14*, 213–228.

(49) Ferris, R. M.; Tang, F. L. M.; Chang, K.-J.; Russell, A. Evidence that the potential antipsychotic agent rimcazole (BW 234U) is a specific, competitive antagonist of *sigma* sites in brain. *Life Sci.* **1986**, *38*, 2329–2337.

(50) Contreras, P. C.; Contreras, M. L.; O'Donohue, T. L.; Lair, C. C. Biochemical and behavioral effects of *sigma* and PCP ligands. *Synapse* **1988**, *2*, 240–243.

Macintosh computer (MAC IICi). The images were set to the same scale using Image software (version 1.4, NIH) and then were combined into a single figure using Aldus Persuasion (Seattle, WA).

Temporal and Regional Distribution Studies. Male CD1 mice (25–30 g) received 2.0 μCi of [^{125}I]PIPAG (specific activity, 204 Ci/mmol) in 0.2 mL of buffered isotonic saline via the tail vein. Nonspecific radiotracer uptake was measured in mice that received haloperidol (1 mg/kg) 5 min before the radiotracer injection. Mice were killed 15–300 min after radioligand administration by cervical dislocation, and they were quickly decapitated. Brains were removed, placed on ice, and dissected. Brain regions were excised, blotted, and weighed. Radioactivity was assayed in an automated γ counter and the percent injected dose/gram of wet tissue was calculated using aliquots of the initial injected dose as a standard. Experiments were conducted on four or five mice per time point. All results shown are the mean ± standard error of the mean (SEM).

In Vivo Competition Studies. Mice were treated as described above for the temporal and regional distribution studies, except that 5 min before injection of radioligand, mice received haloperidol (0.1–1 mg/kg) or unlabeled PIPAG (0.1–5.0 mg/kg) dissolved in saline (0.2 mL) or saline alone (controls). In another experiment, the inhibition values of radiotracer uptake by injections of haloperidol (1 mg/kg), spiperone (1 mg/kg), (±)-NANM (5 mg/kg), and unlabeled PIPAG (5 mg/kg) given 5 min before the radiotracer were compared. Statistical significance of drug treatments were determined by a one-way ANOVA. When the ANOVA revealed a significant effect, means of groups receiving pretreatments of inhibitors were compared to the mean of the saline-pretreated group by Dunnett's test.

Acknowledgment. The authors thank Marigo Stathis, Christine Steinert, Monica Bruckner, and Lionel Raymon for technical assistance. This work was supported in part by U.S. Public Health Service Grants NS 15080 and CA-32845.