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Characterization of Specific Binding Sites for [^{3}H](*d*)-*N*-Allylnormetazocine in Rat Brain Membranes

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SUMMARY

Binding of [^{3}H](*d*)-*N*-allylnormetazocine ([^{3}H](*d*)-NANM) to rat brain membranes is stereospecific, reversible, and saturable (B_{max} = 260 fmol/mg of protein) and manifests moderately high affinity (K_d = 20 nM). The rank order of potency among opioid-benzomorphans and phencyclidine (PCP) analogs for competition for [^{3}H](*d*)-NANM-binding sites is as follows: (*d*)-NANM = PCP-3-OH > (*d*)-cyclazocine > *N*-ethylphenylcyclohexylamine > PCP > (*l*)-cyclazocine = dextrorphan > (*d*/*l*)-ethylketocyclazocine > (*d*/*l*)-bremazocine > (1)-NANM > 1-phenylcyclohexylamine > levorphanol. Other opioid ligands, relatively selective for each of the types of opioid binding sites other than σ, such as morphine (μ), H-Tyr-D-Ala(Me)Phe-NH-CH_2-OH (μ), D-Ala^2-D-Leu^5-enkephalin (δ), tifluadom (κ), and U 50488 (κ) as well as etorphine and naloxone were all unable to compete with [^{3}H](*d*)-NANM for specific binding even at a concentration of 1 μM. Regional distribution studies of [^{3}H](*d*)-NANM-binding sites show high density in the hippocampus, thalamus, hypothalamus, and amygdala and low density in cerebellum and nonfrontal neocortex membranes of the rat brain. These binding sites are very sensitive to protein-modifying enzymes and reagents such as trypsin and *N*-ethylmaleimide and to heat denaturation. These results provide direct biochemical evidence for the existence of distinct (*d*)-NANM-binding sites in rat brain. In addition, this study supports the view that PCP and several of its analogues and the dextrorotatory isomers of psychotomimetic benzomorphans may act at a common recognition site in rat central nervous system.

INTRODUCTION

Based on the variety of pharmacological responses elicited by various classes of opiates in the chronic spinal dog, Martin *et al.* (1, 2) have suggested the existence of three types of opioid receptors. These types (and their prototypic ligands) are μ (morphine), κ (ketocyclazocine), and σ (SKF-10047; NANM[2]). Data from *in vitro* bioassay systems and radioreceptor assays gave evidence for the existence of both a morphine-preferring (μ) binding site which may correspond to the μ receptor proposed by Martin, and an enkephalin-preferring (δ) binding site (3–7). *In vitro* studies have also been used to confirm the existence of distinct κ binding sites in the central and peripheral nervous system (8, 9). It has proved more difficult to provide biochemical evidence for the existence of distinct σ binding sites. Radioreceptor studies have shown considerable cross-reactivity of [^{3}H]-NANM binding with μ, κ, and δ opioid-binding sites in rat brain (10, 11). In a recent study, extremely low levels of [^{3}H](*d*)-NANM specific binding to rat brain membranes were reported (12). Studies of the binding of [^{3}H]cyclazocine have provided some evidence for the existence of high, moderate, and low affinity binding sites which may correspond to μ, κ, and σ sites, respectively (13). The hypothesis of the existence of distinct receptors for NANM is supported, so far, largely by behavioral studies. NANM and related benzomorphans such as pentazocine and cyclazocine produce unique behavioral effects in dog (1, 2), thought to be related to psychotomimetic activity in man (14). Recent, though limited, neuropharmacological studies indicate that some of the behavioral effects elicited by psychotomimetic benzomorphans may be activated preferentially by their dextrorotatory (*d*) isomers (15–17).

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[2] The abbreviations used are: PCP-3-OH, 1-[1-(3-hydroxyphenyl)-cyclohexyl]piperidine; PCP-3-NH_2, 1-[1-(3-aminophenyl)cyclohexyl] piperidine; PC-NH_2, 1-phenylcyclohexylamine; PC-NHEt, *N*-ethyl-phenylcyclohexylamine. PCP-4-Ph-4-OH, 1-(1-phenylcyclohexyl)-4-phenyl-4-piperidinol; PCP, 1-(1-phenylcyclohexyl)piperidine; NANM, *n*-allylnormetazocine, SKF 10047; EKC, ethylketocyclazocine; DAGO, H-Tyr-D-Ala(Me)Phe-NH-CH_2-OH; DADL, D-Ala^2-D-Leu^5-enkephalin; U 50488, *trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl) cyclohexyl]benzeneacetamide; NEM, *N*-ethylmaleimide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TES, 2-{[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino}ethanesulfonic acid.

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Attempts to elucidate the mode of action of PCP, which induces acute psychotic syndromes in man (18), revealed a pharmacological profile very similar to that of NANM in the chronic spinal dog (19). In drug discrimination tests in monkey and rat, generalization occurred between (d)- or (d/l)-NANM and PCP, but not between (l)-NANM and PCP (15). Moreover, in rats, dextrorphan but not levorphanol can produce PCP- or (d)-NANM-like discriminative stimuli (16). The involvement of the opioid system in the effects elicited by PCP and several of its analogs has been suggested on the basis of both *in vivo* and *in vitro* studies. PCP-induced stereotypic behavior, ataxia, and hyperactivity in rats are significantly decreased by the specific opiate antagonist naloxone (20). In addition, we have previously reported that the analgesic effect of PCP in mice is partially reversed by naloxone (21) and its inhibitory effect on the electrically evoked contractions of the guinea pig ileum is also naloxone reversible (22). Opioid receptor-binding studies have indicated that PCP and its analogs compete with opioid ligands for specific binding to rat brain membranes (21–23). These results, together with the demonstration that specific [^{3}H]PCP binding to rat brain membranes (24, 25) is inhibited not only by PCP analogs but also by several benzomorphans (25–27), suggests the existence of common binding sites for benzomorphans and PCP.

The present study was undertaken in order to verify directly the existence of specific binding sites for [^{3}H](d)-NANM in rat central nervous system and to determine the interaction of various opioid ligands and PCP analogs with these sites. Our results indicate moderately high affinity (K_d = 20–30 nM) and levels (B_{max} = 260 fmol/mg of protein) of [^{3}H](d)-NANM specific binding to rat brain membranes. We also provide further biochemical evidence which supports the hypothesis suggesting a common recognition site for the (d) isomers of benzomorphans and PCP and its analogs.

MATERIALS AND METHODS

Crude rat brain membrane fractions were prepared by the method previously described by this laboratory (28). The membrane preparations were stored in 0.32 M sucrose at −70° until needed. For typical binding experiments, duplicate 1-ml samples (0.9–1.1 mg of protein/ml) in 10 mM HEPES-KOH buffer (pH 7.5) containing 1 mM dipotassium-EDTA were incubated for 35 min at 25° with 6 nM [^{3}H](d)-NANM prepared by diluting 1:1 tritium-labeled ligand with unlabeled (d)-NANM. Specific binding was assessed in the absence and presence of 10 μM of either (d)-NANM, (d)-cyclazocine, or PCP-3-OH, all of which resulted in similar levels of nonspecific binding. After incubation, samples were filtered through Whatman GF/B filters presoaked for 40 min with 10 mM HEPES-KOH buffer containing 0.01% polyethyleneimine. Filters were washed three times with 4 ml of ice-cold 10 mM HEPES-KOH buffer and radioactivity remaining on dried filters was determined by scintillation spectroscopy. Membrane protein concentration was determined by the method of Lowry *et al.* (29).

Male Sprague-Dawley rats were purchased from Taconic Farms (Germantown, NY). [^{3}H](d)-NANM (29 Ci/mmol) was purchased from New England Nuclear Corporation, Boston, MA) and this labeled ligand (49 Ci/mmol) as well as unlabeled (d)- and (l)-NANM were generously supplied by Dr. R. Hawks, National Institute on Drug Abuse. The two isomers (d)- and (l)-cyclazocine were kindly donated by Dr. K. Bonnet (N. Y. U. Medical Center, New York). Other compounds used were: (d/l)-EKC (Sterling Winthrop Research Institute, Rensselaer, NY), DADL (Peninsula Laboratories, Inc., San Carlos, CA), DAGO (Reckitt and Coleman, Ltd., Hull, England). (d/l)-Bremazocine and tifluadom were a gift from Dr. D. Römer (Sandoz, Ltd., Basel, Switzerland) and U 50488 was kindly supplied by Dr. M. F. Piercey (Upjohn Company, Kalamazoo, MI). PCP, PCP-3-OH, PCP-3-NH_2, PC-NH_2, PC-NHEt, and PCP-4-Ph-4-OH were synthesized as previously described (30, 31). Chemical analyses for PCP and its analogs were within ±0.3% of the theoretical values.

RESULTS

Specific binding of [^{3}H](d)-NANM to rat brain membranes. In order to determine the optimal conditions needed for the specific binding of [^{3}H](d)-NANM to rat brain membranes, assays were carried out at pH 7.5 in four different buffers, HEPES-KOH, Tris-HCl, K_2HPO_4, and TES-HCl. The best results were obtained in 10 mM HEPES-KOH buffer, where the specific binding of [^{3}H](d)-NANM (6 nM) to rat brain membranes represented 55 ± 5% of the total binding. In typical experiments, total binding of 2552 ± 200 cpm was obtained and nonspecific binding (determined in the presence of 10 μM (d)-NANM or PCP-3-OH) was 1018 ± 120 cpm. Radioactivity adsorbed to GF/B filters, in the absence or presence of 10 μM (d)-NANM or PCP-3-OH, was 693 ± 80 cpm. If radioactivity adsorbed to filter blanks is subtracted from both total and nonspecific binding, the remaining specific binding to rat brain membranes is actually about 80% of total binding. In 10 mM Tris-HCl and K_2HPO_4 buffers or 25 mM TES-HCl buffer, a 30–50% decrease in [^{3}H](d)-NANM specific binding was observed and at a 50 mM concentration of all buffers tested, a 50–70% decrease in specific binding occurred compared to the levels of specific binding obtained in 10 mM HEPES-KOH buffer. All subsequent experiments were therefore conducted in 10 mM HEPES-KOH buffer.

Specific binding of [^{3}H](d)-NANM (6 nM) to rat brain membranes increased linearly with protein concentration in the range of 0.2–1.5 mg/ml. Binding studies of [^{3}H](d)-NANM at concentrations of 1 to 80 nM indicated that specific binding is saturable (Fig. 1A). Scatchard analysis revealed a linear plot with an apparent dissociation constant (K_d) of 20 nM and a maximal number of binding sites (B_{max}) of 260 fmol/mg of protein (Fig. 1B).

Competition binding studies. Various opioid ligands and several PCP analogs were tested for their ability to compete with [^{3}H](d)-NANM for specific binding to rat brain membranes. Results summarized in Table 1 indicate that among the various compounds tested only benzomorphans, dextrorphan, and PCP and several of its analogs were able to compete for specific [^{3}H](d)-NANM binding. The dextrorotatory isomer of NANM and cyclazocine as well as dextrorphan were about 4–15-fold more potent than their levorotatory isomers. The IC_{50} values determined for (d)-NANM and (d)-cyclazocine are 25 and 35 nM, respectively. As shown in Fig. 2, most of specific [^{3}H](d)-NANM binding was inhibited in the range of 10 to 100 nM unlabeled (d)-NANM or PCP-3-OH. (d/l)-EKC and (d/l)-bremazocine, benzomorphan derivatives which display high affinity for κ, μ, and δ opioid binding sites (8), inhibit 50% of [^{3}H](d)-NANM specific binding at a concentration of 200 and

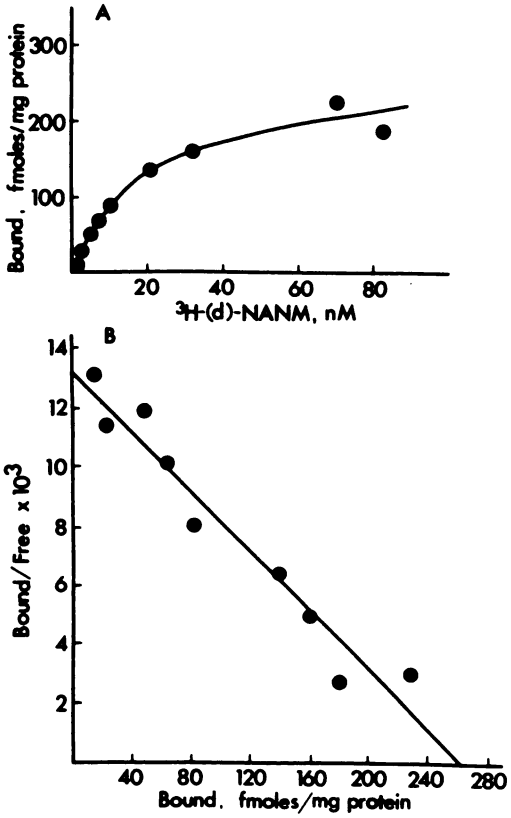


FIG. 1. *Saturation curve (A) and Scatchard plot (B) of [³H](d)-NANM specific binding to rat brain membranes*

Binding of tritiated ligand (1–80 nM) was carried out as described under Materials and Methods. The binding data were analyzed by the program (MED 58) described by Munson and Rodbard (36) and resulted in the selection of the one-site fit as the best fit ($p < 0.05$; $r = 0.88$). Points represent the average values from three experiments.

300 nM, respectively. The levorotatory isomers of the opiate alkaloids morphine, naloxone, etorphine, and nalorphine and the opioid peptides DAGO (μ-agonist) and DADL (δ-preferring agonist) were unable to reduce more than 10–30% of [³H](*d*)-NANM specific binding even at a concentration of 1 μM. Similarly, the relatively selective κ agonists U 50488 and tifluadom were unable to inhibit more than 50% of [³H](*d*)-NANM binding at a concentration of 1 μM.

Among the phencyclidine analogs, PCP-3-OH, which pharmacologically produces PCP-like effects and which was found to be more potent than PCP in the rotarod test (30), was the most potent (IC_{50} = 19 nM) PCP derivative tested for competition for [³H](*d*)-NANM binding (Fig. 2, Table 1). Moreover, PC-NHEt, which is about 10-fold more potent than PC-NH_2 in the rotarod test (30) and in drug discrimination studies (32), is about 10-fold more potent than PC-NH_2 in inhibiting [³H](*d*)-NANM specific binding. The IC_{50} of 90 nM obtained for competition of PCP against [³H](*d*)-NANM is about 2-fold lower than that reported (IC_{50} = 210 nM) for its competition against [³H]PCP (26). The least potent analog in this series is PCP-4-Ph-4-OH, which at a concentration of 1 μM reduces [³H](*d*)-NANM binding by only about 40% (Table 1). Recently, we have characterized this compound to be a μ-selective opioid agonist (33).

TABLE 1

Potency of various drugs in competition with [³H](d)-NANM for specific binding

IC_{50} values for the competition with [³H](*d*)-NANM (6 nM) for specific binding to rat brain membranes were determined from binding studies using eight or nine concentrations (1–10,000 nM) of unlabeled ligand. Pseudo-Hill coefficients (n_H) were computer-generated according to the Hill equation (34) from displacement curves which give the best fit with experimental points. The relative potencies of the various compounds are given, referred to (*d*)-NANM (potency = 1). Data represent the mean ± SE of at least three determinations.

Drug	IC_{50}	n_H	Relative potency
	nM		
Opioid ligands			
(*d*)-NANM	25 ± 3	0.93 ± 0.05	1.00
(*l*)-NANM	310 ± 11	0.77 ± 0.02	0.08
(*d*)-Cyclazocine	35 ± 2	0.91 ± 0.01	0.71
(*l*)-Cyclazocine	150 ± 8	0.75 ± 0.04	0.16
Dextrorphan	180 ± 13	1.01 ± 0.02	0.13
Levorphanol	1000 ± 20		0.02
(*dl*)-EKC	200 ± 7	0.43 ± 0.04	0.12
(*dl*)-Bremazocine	300 ± 9	0.52 ± 0.03	0.08
Etorphine	>1000		
(*l*)-Morphine	>1000		
(*l*)-Naloxone	>1000		
Nalorphine	>1000		
U 50488	>1000		
Tifluadom	>1000		
DAGO	>1000		
DADL	>1000		
Phencyclidines			
PCP	90 ± 5	0.88 ± 0.02	0.27
PCP-3-OH	19 ± 1	0.85 ± 0.04	1.31
PCP-3-NH_2	700 ± 30	0.86 ± 0.01	0.03
PC-NHEt	40 ± 3	1.10 ± 0.03	0.62
PC-NH_2	350 ± 22	0.73 ± 0.05	0.07
PCP-4-Ph-4-OH	>1000		

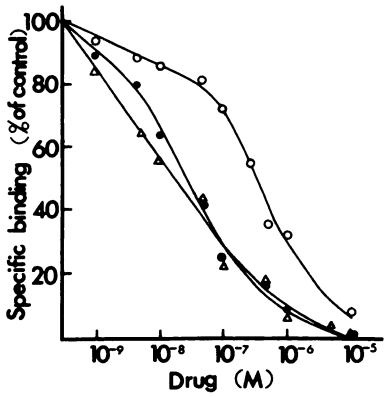


FIG. 2. *Inhibition of [³H](d)-NANM specific binding to rat brain membranes by various compounds*

The ability of (*d*)-NANM (●), (*l*)-NANM (○), and PCP-3-OH (△) to compete with [³H](*d*)-NANM (6 nM) for specific binding was determined by using eight concentrations of unlabeled ligand. Control specific binding was 1580 ± 240 cpm/ml. Each displacement curve represents the mean of three assays.

Binding kinetics. Kinetic analysis of [^{3}H](*d*)-NANM specific binding was carried out. The half-time for association of 6 nM of [^{3}H](*d*)-NANM was 2.5 ± 0.3 min (at 25°) and the reaction was completed within 20 min (Fig. 3A). The second order constant calculated for the association rate (Fig. 3B) was 3.1×10^6 M^{-1} min^{-1}. The half-time for dissociation of specifically bound [^{3}H](*d*)-NANM (6 nM) was 6.5 ± 0.5 min (Fig. 4) and the first order rate constant for dissociation was 0.106 min^{-1}. When the equilibrium dissociation constant (K_d) was calculated from the kinetic data ($k_d/k_a = K_d$), the value of 34 nM was obtained, which is in satisfactory agreement with results (20 nM) obtained from equilibrium binding experiments.

Effects of various reagents. Further characterization of (*d*)-NANM-binding sites was carried out by determining the effects of protein-modifying reagents, temperature, and various cations on [^{3}H](*d*)-NANM specific binding. Results are summarized in Table 2. The sulfhydryl reagent NEM (0.5 mM), and the proteolytic enzyme trypsin (20 μg/ml) decreased [^{3}H](*d*)-NANM specific binding by 61 ± 5 and 73 ± 10%, respectively. Preincubation of rat brain membranes at 60° for 20 min resulted in a 72 ± 3% decrease in specific binding. The various salts tested, at a concentration of 100 mM, decrease by 40–75% the specific binding (Table 2). In the presence of GTP (100 μM) alone, no significant change in specific binding was observed; however in the presence of both GTP and NaCl (20 mM), a 46 ± 1% decrease in specific binding was observed, which is greater than with this concentration of NaCl alone.

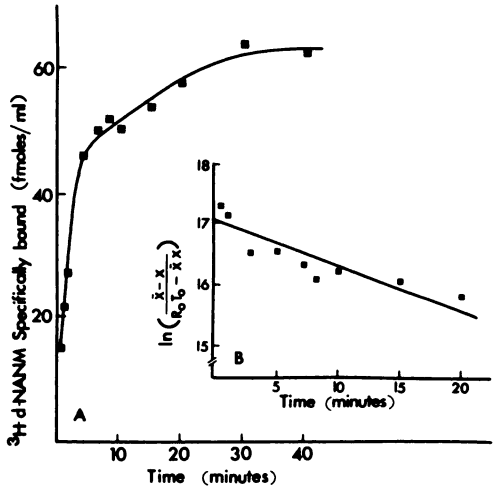


FIG. 3. *Kinetics of specific binding of [^{3}H](d)-NANM to rat brain membranes*

A, association time course of specifically bound [^{3}H](*d*)-NANM (6 nM) to rat brain membranes at 25°. B, the second order rate constant of association was calculated from:

$$K_2 = \frac{1}{t}\,\frac{\bar{x}}{x^{-2} - R_0 \cdot T_0}\,\ln\frac{\bar{x} - x}{R_0 \cdot T_0 - \bar{x}x} + \ln\frac{R_0 \cdot T_0}{\bar{x}}$$

as formulated by Maelichke *et al.* (37). The concentration of [^{3}H](*d*)-NANM-binding sites (R_0) equaled 0.26 pmol/mg of protein, and the tritiated ligand concentration (T_0) was 6 nM. The equilibrium concentration of binding site-ligand complex ($\bar{x}$) was 6.9×10^{-11} M. Data represent the mean of three assays.

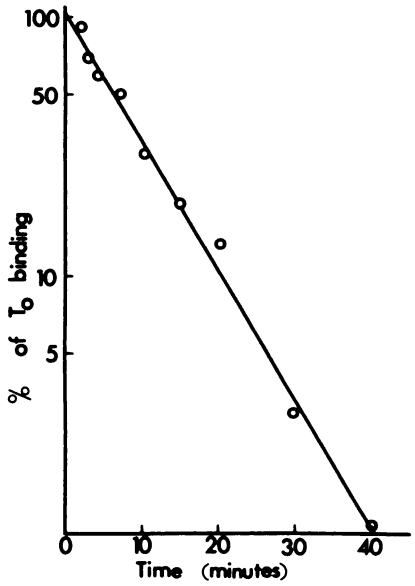


FIG. 4. *Dissociation of specifically bound [^{3}H](d)-NANM from rat brain membranes*

Binding of tritiated ligand (6 nM) was carried out at 25° for 35 min. At time 0 (T_0), a 1000-fold excess of unlabeled drug was added to the homogenates and specific binding remaining in aliquots of 1-ml samples, at indicated time intervals, was determined and plotted as log of percentage of T_0 binding against time. Data represent the mean of three assays whose points varied less than 10% from the mean.

TABLE 2

Effects of various reagents on [^{3}H](d)-NANM specific binding to rat brain membranes

Specific binding of [^{3}H](*d*)-NANM (6 nM) to rat brain membranes was assessed in control preparations (1500 ± 200 cpm/ml) and in samples treated with protein-modifying reagents (20 min at 30°) prior to the binding assay. In the case of NEM, unreacted reagent was destroyed by addition of dithiothreitol (5 mM). The various salts tested were added to the reaction mixture just before the binding assay. Data represent the mean ± SE of three assays.

Reagent	Concentration	Specific binding
		% control
NEM	0.5 mM	39 ± 5
Trypsin	20 μg/ml	27 ± 10
GTP	100 μM	103 ± 2
GTP	100 μM	
+NaCl	20 mM	31 ± 3
NaCl	20 mM	54 ± 1
	100 mM	36 ± 4
KCl	100 mM	59 ± 5
LiCl	100 mM	24 ± 3
$MgCl_2$	5 mM	28 ± 2
$CaCl_2$	5 mM	26 ± 2
$MnCl_2$	5 mM	22 ± 6
60°/20 min		28 ± 3

Regional distribution. Binding of [^{3}H](*d*)-NANM (6 nM) was carried out in membrane preparations derived from various regions of rat brain. As indicated in Table 3, the highest levels of [^{3}H](*d*)-NANM specific binding were found in the hippocampus, thalamus, hypothalamus, and amygdala. Moderate levels of binding were found in the frontal cortex and caudate and low levels in

TABLE 3

Regional distribution of [^{3}H](d)-NANM specific binding in rat brain

Various regions of rat brain were dissected according to König and Klippel (35) and prepared as described under Materials and Methods. Binding assays were carried out in 1 ml of membrane preparation, containing 0.9–1.1 mg of protein/ml. In each experiment, specific binding of 6 nM [^{3}H](*d*)-NANM was determined in regions pooled from eight to 10 rat brains. The mean ± SE of three to four experiments is represented.

Region	Specific binding
	fmol/mg protein
Hippocampus	101 ± 8
Thalamus	90 ± 2
Hypothalamus	88 ± 10
Amygdala	87 ± 7
Pons/medulla	76 ± 6
Midbrain	65 ± 6
Frontal cortex	53 ± 5
Caudate	51 ± 2
Cerebellum	35 ± 6
Nonfrontal cortex	27 ± 4

the cerebellum and nonfrontal neocortex (i.e., all neocortex excluding frontal cortex).

DISCUSSION

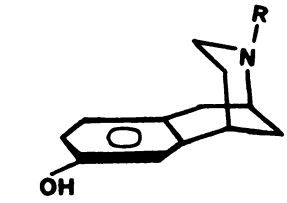
Results presented in this study provide direct biochemical evidence for the existence of distinct binding sites for the dextrorotatory isomer of *N*-allylnormetazocine in rat brain membranes. Specific binding of [^{3}H](*d*)-NANM is reversible and saturable and exhibits fairly high affinity, thus fulfilling some of the criteria for drug-receptor interactions. Scatchard analysis reveals a B_{max} of 260 fmol/mg of protein and a K_d of 20 nM. The linearity of the plot, obtained within a concentration range of 1 to 80 nM, suggests an interaction with a homogenous population of binding sites. Both kinetic and competitive studies indicate that [^{3}H](*d*)-NANM specific binding is reversible. The $t_{1/2}$ for dissociation is 6.5 ± 0.5 min and complete dissociation occurs within 30 min. The K_d value calculated from kinetic studies is in good agreement with that found from equilibrium studies.

The involvement of protein components in [^{3}H](*d*)-NANM binding is suggested by the following findings. (a) A large decrease (72%) in specific binding occurs following treatment of rat brain membranes with trypsin or exposure to heat (60°). (b) The decrease of [^{3}H](*d*)-NANM specific binding observed following NEM treatment of rat brain membranes suggests the presence of SH group(s) in or near the binding site.

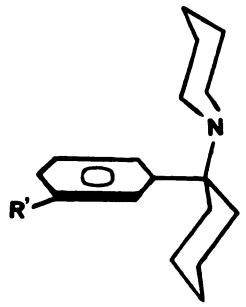
Our ability to demonstrate rather high affinity and levels of binding of [^{3}H](*d*)-NANM in this study became possible due to the use of low ionic strength buffer. HEPES-KOH (10 mM) proved to be the best. Therefore, the decrease in specific binding observed in the presence of 100 mM concentrations of various salts tested (NaCl, KCl, and LiCl) could be due to the increase in ionic strength. However, $MgCl_2$, $MnCl_2$, and $CaCl_2$, at a concentration of 5 mM each, reduce the binding by 72–78% compared to control values, which could reflect specific cationic inhibition. In a recent study where binding assays were carried out in 50 mM Tris-HCl buffer, only rather low levels of [^{3}H](*d*)-NANM specific binding were reported (12).

The highest density of [^{3}H](*d*)-NANM-binding sites is present in the hippocampus, thalamus, hypothalamus, and amygdala, regions which are components of the limbic system or associated with this system and which possess moderate to high levels of classical opioid receptors (4). Whereas high levels of [^{3}H]PCP specific binding are present also in the hippocampus (26), only moderate levels of [^{3}H]DADL and [^{3}H]naloxone binding were found in this region of the rat brain (4).

Further characterization of [^{3}H](*d*)-NANM-binding sites revealed that these sites are clearly distinguishable from μ, δ, and κ binding sites. The relatively selective opioid ligands, morphine (μ), DAGO (μ), DADL (moderately δ selective), U 50488 (κ), and tifluadom (κ) were unable to inhibit more than 20–30% of [^{3}H](*d*)-NANM specific binding to rat brain membranes even at a concentration of 1 μM. However, benzomorphans (NANM, cyclazocine, EKC, and bremazocine), dextrorphan, and PCP and its analogs display rather high (IC_{50} = 19–70 nM) to moderate (IC_{50} = 90–300 nM) affinity for [^{3}H](*d*)-NANM-binding sites. Within the group of benzomorphans, variations in affinity are due to the stereochemistry, i.e., the dextrorotatory isomers have higher affinity than the levorotatory. Although the stereospecificity of [^{3}H](*d*)-NANM-binding sites is not as high as that observed for the μ-opioid binding sites, (*d*)-NANM (IC_{50} = 25 nM) is nevertheless 12-fold more potent than (*l*)-NANM, while (*d*)-cyclazocine (IC_{50} = 35 nM) and



Benzomorphan



R'= H ; PCP

R'= OH ; PCP-3-OH

FIG. 5. *The structures of benzomorphan, PCP, and PCP-3-OH*

Note that the planes of the aromatic ring and the nitrogen atom in benzomorphan are superimposable with those in PCP. The distances, as measured in Dreiding stereomodels, between the nitrogen atom and the center of the π electrons in the benzomorphan structure is 3.9 Å and in PCP is 3.2 Å.

dextrorphan (IC_{50} = 180 nM) are 4–5-fold more potent than their levorotatory isomers in displacing [^{3}H](*d*)-NANM specific binding.

Fairly low concentrations of PCP analogs are effective in competing with [^{3}H](*d*)-NANM binding. However, an exception is PCP-4-Ph-4-OH, which displays extremely low affinity for these sites (Table 1). Recently, we have shown that this analgesic, derived from PCP (31), is a highy selective μ-opioid agonist (33). The most potent PCP analog for competition with [^{3}H](*d*)-NANM for receptor binding is PCP-3-OH (IC_{50} = 19 nM) which is 5-fold more potent than PCP. PC-NHEt (IC_{50} = 40 nM) is 10-fold more potent than PC-NH_2. These results correspond rather well with *in vivo* pharmacological studies indicating that PCP-3-OH is more potent than PCP as determined in the rotarod test (30). Similarly, PC-NHEt was found to be 10-fold more potent than PC-NH_2 in both the rotarod test (30) and drug discriminative studies (32). These results provide not only direct evidence for the interaction of PCP and these analogs with (*d*)-NANM sites, but may also suggest the pharmacological relevance of these binding sites.

Previous studies dealing with the demonstration of PCP specific binding sites in rat brain (24–27) have suggested the existence of common binding sites for PCP and benzomorphans (25–27), which may correspond to the σ opioid-binding sites proposed by Martin *et al.* (1, 2). This view was supported by findings which indicate the opiate-like effects induced by PCP and several analogs as determined by *in vivo* and *in vitro* studies (21, 22, 31). The involvement of σ opioid-binding sites in the mediation of some of these effects has been proposed (21). In the present study, with the availability of tritium-labeled (*d*)-NANM, it became possible to provide direct evidence for the existence of specific binding sites for (*d*)-NANM, which may indeed correspond to the σ binding sites. In addition, this study supports the view that these binding sites may overlap with PCP sites.

Although PCP is structurally different from benzomorphans, Dreiding stereomodels of PCP suggest that this molecule can easily rearrange to the "T" shape (Fig. 5), which is common to morphinans and benzomorphans. Furthermore, these models show that the functional moieties, i.e., nitrogen atom and aromatic ring of PCP and benzomorphans, are superimposable (Fig. 5). The distances between the nitrogen atom and aromatic ring in PCP and benzomorphans are 3.2 and 3.9 Å, respectively, as measured in Dreiding stereomodels. In PCP-3-OH, the most potent analog in the PCP series, the hydroxyl group at the *meta* position of the aromatic ring, is oriented in space in the same position as in the benzomorphans (Fig. 5). Moreover, since PCP-3-OH is about 40-fold more potent than PCP-3-NH_2 in competing with [^{3}H](*d*)-NANM for specific binding, it may suggest the necessity of the phenolic moiety, as with opiate benzomorphans, for interaction with the binding site. Together, these observations suggest that benzomorphan and PCP molecules may fulfill some similar space requirements which permit interaction with a common recognition site.

The physiological significance of [^{3}H](*d*)-NANM specific binding sites has yet to be ascertained. Data presented in this study provide some biochemical evidence for the existence of a distinct type of binding site which may be involved in the mode of action of psychotomimetic drugs.

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