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Characterization of Opioid, σ, and Phencyclidine Receptors in the Neuroblastoma-Brain Hybrid Cell Line NCB-20

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SUMMARY

Opioid, σ, and phencyclidine (PCP) receptors were characterized in the mouse neuroblastoma—Chinese hamster brain hybrid cell line NCB-20. Quantitative receptor assays under equilibrium binding conditions with highly specific radioligands demonstrated the presence of δ, but not μ or κ, opioid receptors on NCB-20 cell membranes. NCB-20 cells were shown to possess two distinct sites specific for σ opioids and PCP derivatives. One site was labeled by (+)-[^{3}H]N-allylnormetazocine [(+)-[^{3}H]SKF-10,047] (K_d = 69 nM; B_{max} = 4100 fmol/mg of protein). The rank order of potency of drugs at this site was (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine [(+)-3-PPP] > haloperidol > (+)-SKF-10,047 > (±)-ethylketocyclazocine > (±)-bremazocine > *N*-[1-(2-thienyl) cyclohexyl]piperidine (TCP) > dexoxadrol. This site is similar in its ligand selectivity to the haloperidol-sensitive σ receptor of rat brain. The other site was labeled by the potent phencyclidine derivative [^{3}H]TCP (K_d = 335 nM; B_{max} = 9300 fmol/mg of protein). This density is equivalent to approximately 60,000 sites/cell. The rank order of potency of drugs at this site was TCP > (+)-3-PPP > PCP > dexoxadrol > haloperidol > cyclazocine > levoxadrol > (+)-SKF-10,047; μ and δ ligands were inactive. This site is similar to the rat brain PCP receptor. The NCB-20 cell line is the only cultured cell line that has been demonstrated to have PCP receptors.

The actions of opiates and opioid peptides on neuronal tissue are mediated by the μ, δ, and κ opioid receptors and PCP and σ receptors. These receptors exhibit distinct ligand selectivity patterns, have unique neuroanatomical distribution patterns, and can be distinguished by their different physiological and behavioral effects (for reviews see Refs. 1 and 2). Recently, considerable interest has focused on the receptors for σ opioids. The "σ opiate" receptor was originally postulated to be the site at which the psychotomimetic and stimulatory effects of *N*-allylnormetazocine (SKF-10,047), cyclazocine, and related benzomorphan opioids are mediated (3). Considerable evidence now indicates that (+)-SKF-10,047 interacts with two binding sites in mammalian brain, neither of which is naloxone-sensitive (see Ref. 4). The phencyclidine receptor mediates the unique psychotomimetic as well as other behavioral effects induced by PCP derivatives, σ opioids, and dioxalanes (for a review see Ref. 4). It can be labeled by [^{3}H]PCP, by the more potent and specific PCP derivative [^{3}H]TCP (5, 6), by [^{3}H] cyclazocine in the presence of μ and κ blockers (7), and by (+)-[^{3}H]SKF-10,047 (8, 9). Evidence from electrophysiological (10–12), neurochemical (13, 14), and biochemical (15–17) studies suggests that the PCP receptor and the *N*-methyl-D-aspartate (NMDA) - type glutamate receptor are components of a single supramolecular complex.

The other σ opioid binding site is relatively insensitive to PCP but is very sensitive to the butyrophenone neuroleptic haloperidol and to the putative dopamine autoreceptor ligand (+)-3-PPP. This site can be labeled with (+)-[^{3}H]SKF-10,047 (8, 9) and with (+)-[^{3}H]3-PPP (9). To date, no specific behavioral or physiological function has been demonstrated for the haloperidol-sensitive σ receptor, and thus its role in mediating some component of σ opioid psychotropic action is unknown.

An important objective of the present study was to identify a clonal cell line containing PCP receptors. The mouse neuroblastoma-Chinese hamster brain hybrid NCB-20 cells were previously shown to have δ opioid receptors and benzomorphan-specific sites (18, 19) (possibly κ or "σ opioid" binding sites) on their cell membranes. In the present study, highly specific radioligands were used to label opioid, PCP, and σ receptors in quantitative receptor assays of NCB-20 cells.

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ABBREVIATIONS: PCP, phencyclidine; TCP, *N*-[1-(2-thienyl)cyclohexyl]piperidine; SKF-10,047, *N*-allylnormetazocine; 3-PPP, 3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine; EKC, ethylketocyclazocine; NMDA, *N*-methyl-D-aspartate; D-AP5, D-aminophosphonovaleric acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid.

This study demonstrates the presence of δ, PCP, and σ binding sites in this cell line. The NCB-20 cell line is the only clonal cell line known to have receptors suggesting that it will prove to have NMDA receptors as well.

Experimental Procedures

Materials. [^{3}H]TCP (52.9 Ci/mmol), (+)-[^{3}H]SKF-10,047 (43.3 Ci/mmol), [^{3}H][2-D-penicillamine,5-D-penicillamine]-enkephalin (41.4 Ci/mmol), (−)-[^{3}H]bremazocine (30.0 Ci/mmol), [^{3}H]D-Ala^2,*N*-MePhe^4,Gly-ol^5-enkephalin (60 Ci/mmol), (−)-[^{3}H]EKC (55 Ci/mmol), and [^{3}H]U69,593 (42.1 Ci/mmol) were purchased from New England Nuclear (Boston, MA); D-AP5 was purchased from Cambridge Research Biochemicals (Valley Stream, NY). Levorphanol was obtained from Hoffman-LaRoche (Nutley, NJ). U-50,488H and U-69,593 were purchased from Upjohn Diagnostics (Kalamazoo, Michigan). (±)-Ethylketocyclazocine, (−)-EKC, and cyclazocine were obtained from Sterling-Winthrop Research Institute (Renssalear, NY). [2-D-Penicillamine,5-D-penicillamine]-enkephalin, and D-Ala^2, *N*-MePhe^4,Gly-ol^5-enkephalin were purchased from Peninsula Laboratories (San Carlos, CA). TCP, PCP, dexoxadrol, levoxadrol, (+)-SKF-10,047, and (−)-SKF-10,047 were furnished by the National Institute of Drug Abuse. (+)-3-PPP and (−)-3-PPP were the generous gifts of Dr. Menek Goldstein, New York University School of Medicine (New York, NY). Bremazocine was the generous gift of Dr. H. B. A. Welle of ACF Chemiefarmon, The Netherlands. All other reagents were obtained from normal commercial sources.

Cell culture and membrane preparation. The mouse neuroblastoma-Chinese hamster brain hybrid cell line NCB-20 was the generous gift of Dr. Richard Miller, University of Chicago. Cells were grown in monolayer in Dulbecco's modified Eagle medium supplemented with 5% fetal bovine serum (GIBCO, Grand Island, NY) and garamycin (50 μg/ml) on 100-mm^2 Falcon tissue culture dishes in a humidified atmosphere of 5% CO_2 and 95% air. Cells were harvested at confluency, resuspended in 33 volumes of ice-cold 50 mM Tris·HCl, pH 7.4, and swelled on ice for 30 min before homogenizing with a Brinkman Polytron. The resulting homogenate was centrifuged at 30,000 × *g* for 15 min at 4°. Resuspension and centrifugation of the pellet were repeated. Pellets were resuspended in 33 volumes of 50 mM Tris·HCl, pH 7.4, and incubated (37° for 30 min) in order to facilitate the dissociation of endogenous ligand from the receptors. After centrifugation at 30,000 × *g* for 10 min at 4°, pellets were resuspended in assay buffer to a final protein concentration of 0.5–0.7 mg/ml. Membrane protein concentration was determined by the method of Lowry *et al.* (20).

Receptor binding assays. Radioligand binding was as described previously (21). For opioid receptor binding assays, samples (0.5 ml) of NCB-20 cell membranes were incubated with [^{3}H]D-Ala^2,*N*-MePhe^4,Gly-ol^5-enkephalin (μ-specific opioid), [^{3}H][2-D-penicillamine,5-D-penicillamine]-enkephalin (δ-specific opioid), [^{3}H]U-69,593 (κ-specific opioid), or (−)[^{3}H]-bremazocine (universal opioid ligand) in 50 mM Tris·HCl buffer, pH 7.4, in the absence or presence of levorphanol (10 μM) at 4° for 45 min [except in the case of δ receptor assays, which were carried out in the absence or presence of D-Ala^2,D-Leu^5-enkephalin (10 μM) for 3 hr]. In the case of κ receptor assays, binding was carried out using either [^{3}H]U-69,593 [which may interact with only a subpopulation of κ receptors (22)] or (−)-[^{3}H]bremazocine in the presence of 100 nM D-Ala^2,*N*-MePhe^4,Gly-ol^5-enkephalin and 1 μM D-Ala^2-D-Leu^5-enkephalin to block μ and δ receptors, respectively, and to direct the binding of the radioligand to κ receptors. For PCP and σ receptor assays, samples (0.5 ml) were incubated with [^{3}H]TCP (specific PCP receptor ligand) in the presence or absence of TCP (100 μM) or with (+)-[^{3}H]SKF-10,047 in the presence or absence of (+)-SKF-10,047 (10 μM) in 5 mM Tris·HCl buffer, pH 7.4 (4° for 45 min). For all assays, free radioligand was separated from bound ligand by filtration under reduced pressure through Whatman GF/B filters, which in the case of [^{3}H]U-69,593, [^{3}H]TCP, and (+)-[^{3}H]SKF-10,047 had been previously soaked in 0.3% polyethyleneimine. Filters were washed with three consecutive 4-ml aliquots of ice-cold assay buffer. Filters were transferred to 4 ml of Aquasol (New England Nuclear) and assayed by liquid scintillation counting at a counting efficiency of approximately 50%. Binding affinities and capacities were calculated by Scatchard analysis utilizing computer-assisted linear regression analysis. All experiments were performed in triplicate and replicated a minimum of three times.

Results

Quantitative receptor measurements. Equilibrium binding of the δ-specific ligand [^{3}H][2-D-penicillamine,5-D-penicillamine]-enkephalin to membranes of NCB-20 cells was monophasic, of high affinity, and saturable (Fig. 1A). Scatchard analysis of this binding revealed a linear plot (Fig. 1A, *inset*). Computer-assisted linear regression analysis afforded a best fit for a single binding site with an affinity (K_d) of 7.7 nM and a receptor density (B_{max}) of 460 fmol/mg of protein. Specific binding was undetectable in the case of the μ-specific ligand [^{3}H]D-Ala^2,*N*-MePhe^4,Gly-ol^5-enkephalin. The receptor density determined for the universal opioid ligand (−)-[^{3}H]bremazocine (Fig. 1B) was identical to that obtained for [^{3}H][2-D-

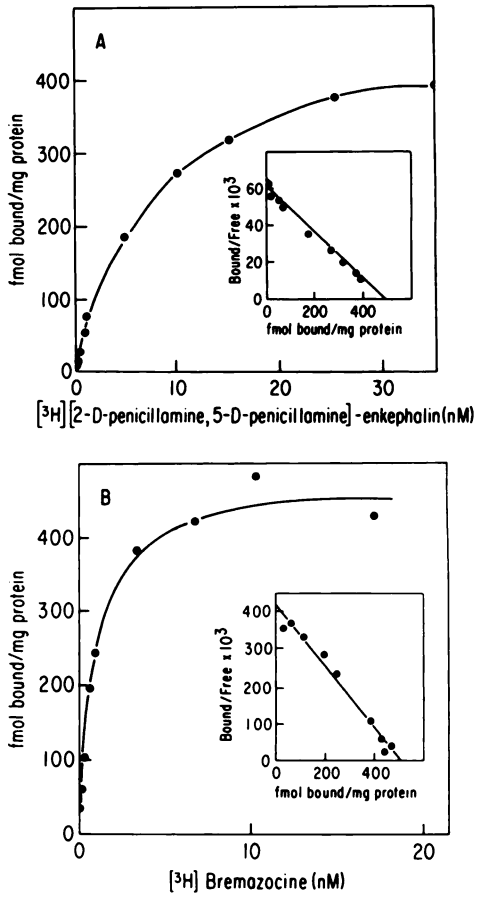

Fig. 1. Saturation plots and Scatchard plots (*insets*) of specific binding to opioid receptors of NCB-20 cell membranes. Membranes (0.6 mg of protein/ml) were incubated with [^{3}H][2-D-penicillamine,5-D-penicillamine]-enkephalin (0.15–25 nM) (4° for 3 hr) (A) or (−)-[^{3}H]bremazocine (0.15–22 nM) (4° for 45 min) (B) in 50 mM Tris·HCl buffer, pH 7.4. Details of the receptor assay are presented under Materials and Methods. The Scatchard plots were fit by a straight line using linear regression analysis. These data are from a representative experiment that was performed in triplicate and replicated a minimum of three times.

penicillamine,5-D-penicillamine]-enkephalin. Scatchard analysis of (−)-[^{3}H]bremazocine binding (Fig. 1B, *inset*) revealed a linear plot, indicating an apparent single class of sites (K_d = 1.3 nM, B_{max} = 470 fmol/mg of protein). Specific binding of (−)-[^{3}H]bremazocine in the presence of saturating concentrations of μ and δ ligands was undetectable. Competition analyses (Fig. 2) showed that the δ ligand D-Ala^2-D-Leu^5-enkephalin displaces 100% of (−)-[^{3}H]bremazocine binding (which in the absence of μ receptors represents δ binding) to NCB-20 cell membranes (IC_{50} = 2.9 ± 0.7 nM) as does the δ-specific ligand [2-D-penicillamine,5-D-penicillamine]-enkephalin (IC_{50} = 3.1 ± 1.0 nM). The relatively specific κ ligand U-50,488H was only weakly active in displacing (−)-[^{3}H]bremazocine binding (IC_{50} = 10.8 ± 3.4 μM), as was the κ-specific ligand U-69,593 (IC_{50} = 5.9 ± 0.8 μM). Similar results were obtained for the ligand (−)-[^{3}H]EKC (data not shown). In addition, [^{3}H]U-69,593 did not exhibit specific binding to NCB-20 cell membranes. Together, these data indicate the presence of δ opioid receptors, but not μ or κ opioid receptors, in the NCB-20 cell line.

Scatchard analysis of (+)-[^{3}H]SKF-10,047 binding to NCB-20 cell membranes (Fig. 3A) revealed an apparent single class of sites with a binding affinity of 69 nM and receptor density of 4100 fmol/mg (Fig. 3A, *inset*). It should be noted that when the titration experiment was carried out to include concentrations of (+)-[^{3}H]SKF-10,047 exceeding 250 nM, the Scatchard plot and saturation curve appeared biphasic. However, increasing levels of nonspecific binding made it difficult to reliably interpret the data. (+)-3-PPP was the most potent inhibitor (IC_{50} = 24 nM) of binding of (+)-[^{3}H]SKF-10,047 (Table 1). Haloperidol and (+)-SKF-10,047 were approximately as potent as (+)-3-PPP at this site. TCP, PCP, and U-50,488H were somewhat less potent. Stereoselectivity was demonstrated at this site not only in that dexoxadrol was 2-fold more potent than levoxadrol, but also in that (+)-SKF-10,047 was 20-fold more potent than (−)-SKF-10,047. D-Ala^2,*N*-MePhe^4,Gly-ol^5-enkephalin, normorphine, and D-Ala^2,D-Leu^5-enkephalin did not displace (+)-[^{3}H]SKF-10,047 at concentrations up to 20 μM. Although (±)-EKC was a very potent inhibitor of (+)-[^{3}H]SKF-10,047 binding (IC_{50} = 58 nM), (−)-EKC did not displace at concentrations up to 20 μM.

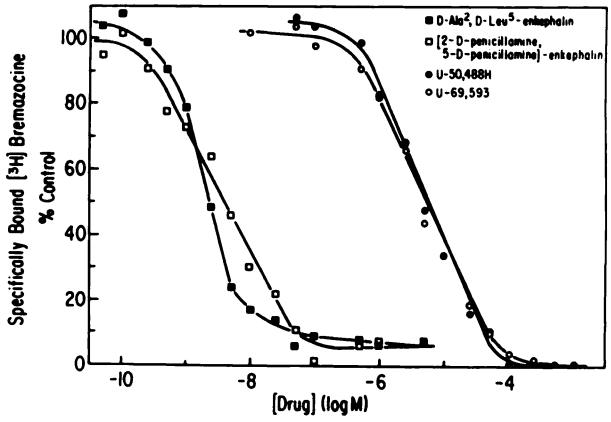

Fig. 2. Competition analyses of (−)-[^{3}H]bremazocine binding to NCB-20 cell membranes. Membranes (0.6 mg of protein/ml) were incubated with (−)-[^{3}H]bremazocine (1 nM) and various concentrations of U-50,488H (●), U69,593 (○), D-Ala^2,D-Leu^5-enkephalin (■), or [2-D-penicillamine,5-D-penicillamine]-enkephalin (□) in 50 mM Tris·HCl buffer, pH 7.4 (4° for 45 min). Details of the receptor assay are presented under Materials and Methods. The data are the mean values of a representative experiment that was performed in triplicate and replicated a minimum of three times.

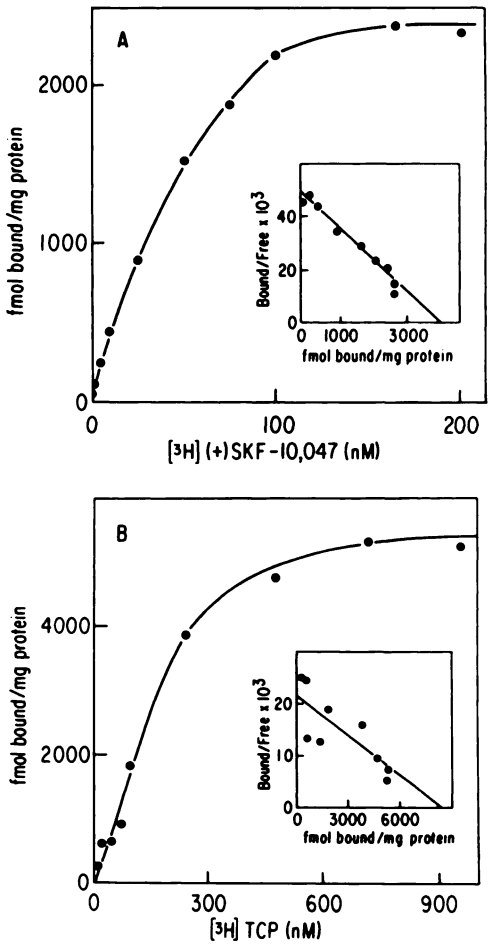

Fig. 3. Saturation plots and Scatchard plots (*insets*) of specific binding to PCP and σ receptors of NCB-20 cell membranes. Membranes (0.6 mg of protein/ml) were incubated with [^{3}H]TCP (0.01–1 μM) (A) or (+)-[^{3}H]SKF-10,047 (1–300 nM) (B) in 5 mM Tris·HCl buffer, pH 7.4 (4° for 45 min). Details of the receptor assay are presented under Materials and Methods. The Scatchard plots were fit by a straight line using linear regression analysis. These data are from a representative experiment that was performed in triplicate and replicated a minimum of three times.

Equilibrium binding of the PCP receptor-specific ligand [^{3}H]TCP to NCB-20 cell membranes was monophasic and saturable with respect to radiolabeled ligand concentration (Fig. 3B) in 100% of passages tested (n = 9). Binding to this site was protease- and heat-sensitive (data not shown). Moreover, no specific binding of this ligand to membranes from the rat glioma cell line C6 was observed. When [^{3}H]TCP binding to NCB-20 cell membranes was analyzed by Scatchard analysis (Fig. 3B, *inset*), it revealed a linear plot (K_d = 335 nM, B_{max} = 9300 fmol/mg of protein). The receptor density corresponds to approximately 60,000 sites per cell. TCP was the most potent displacer of specifically bound [^{3}H]TCP (IC_{50} = 0.2 μM) (Table 2). PCP was almost as potent (IC_{50} = 0.3 μM), and haloperidol exhibited moderate affinity (IC_{50} = 0.93 μM). However, (+)-SKF-10,047 was almost as low in the rank order of potency (IC_{50} = 2.3 μM) at this site as its stereoisomer (−)-SKF-10,047 (IC_{50} = 3.2 μM). The benzomorphan (−)-EKC did not displace [^{3}H]TCP at concentrations up to 20 μM, nor did D-Ala^2,*N*-MePhe^4,Gly-ol^5-enkephalin,D-Ala^2,D-Leu^5-enkephalin, [2-D-penicillamine,5-D-

TABLE 1

Relative potencies of drugs in inhibiting specific (+)-[^{3}H]SKF-10,047 binding to NCB-20 cell membranes

NCB-20 cell membranes (0.7 mg of protein/ml) were incubated in 5 mM Tris·HCl buffer, pH 7.4 (4° for 45 min) in the presence of 10 nM (+)-[^{3}H]SKF-10,047 and drugs as indicated. Details of the receptor assay are described under Materials and Methods. IC_{50} values were calculated from linear regression analyses of log-logit plots constructed using a minimum of eight concentrations of each drug. Data reported are means ± standard errors of the averaged values from a minimum of three experiments, each performed in triplicate. Control binding for 10 nM (+)-[^{3}H]SKF-10,047 was 1434 cpm specifically bound per sample. This represents 75% of total binding.

	Ligand	IC_{50}	Relative potency[a]
		μM	
(1)	(+)-3-PPP	0.024 ± 0.006	1.92
(2)	Haloperidol	0.030 ± 0.007	1.53
(3)	(+)-SKF-10,047	0.046 ± 0.015	1.00
(4)	(±)-EKC	0.058 ± 0.006	0.79
(5)	(±)-Bremazocine	0.12 ± 0.01	0.38
(6)	TCP	0.26 ± 0.03	0.18
(7)	PCP	0.31 ± 0.01	0.15
(8)	Dexoxadrol	0.34 ± 0.06	0.14
(9)	U-50,488H	0.37 ± 0.04	0.12
(10)	Cyclazocine	0.53 ± 0.11	0.09
(11)	Levoxadrol	0.65 ± 0.09	0.07
(12)	(−)-SKF-10,047	1.0 ± 0.1	0.05
(13)	(−)-3-PPP	1.1 ± 0.1	0.04
(14)	[2-D-penicillamine,5-D-penicillamine]-enkephalin	11.7 ± 6.08	0.004
(15)	D-Ala^2,*N*-MePhe^4,Gly-ol^5-enkephalin	>50.0	0
(16)	D-Ala^2,D-Leu^5-enkephalin	>50.0	0
(17)	Normorphine	>50.0	0
(18)	(−)-EKC	>50.0	0

[a] Potencies relative to (+)-SKF-10,047.

TABLE 2

Relative potencies of drugs in inhibiting specific [^{3}H]TCP binding to NCB-20 cell membranes

NCB-20 membranes (0.7 mg of protein/ml) were incubated in 5 mM Tris·HCl buffer, pH 7.4, (4° for 45 min) in the presence of 50 nM [^{3}H]TCP and drugs as indicated. Details of the receptor assay are described under Materials and Methods. IC_{50} values were calculated from linear regression analyses of log-logit plots constructed using a minimum of eight concentrations of each drug. Data reported are the means ± standard errors of the averaged values from a minimum of three experiments, each performed in triplicate. Control binding for 50 nM [^{3}H]TCP was 6548 cpm specifically bound per sample. This represents 76% of total binding.

Ligand	IC_{50}	Relative potency[a]
	μM	
TCP	0.22 ± 0.04	1.00
(+)-3-PPP	0.27 ± 0.17	0.81
PCP	0.34 ± 0.03	0.65
3-Amino-PCP	0.57 ± 0.21	0.39
U-50,488H	0.61 ± 0.16	0.36
Dexoxadrol	0.90 ± 0.13	0.24
Haloperidol	0.93 ± 0.28	0.24
Cyclazocine	1.7 ± 0.3	0.13
Levoxadrol	2.3 ± 0.4	0.10
(+)-SKF-10,047	2.34 ± 0.2	0.10
(−)-SKF-10,047	3.2 ± 1.2	0.07
(±)-Bremazocine	5.9 ± 2.56	0.04
(−)-3-PPP	5.9 ± 2.4	0.04
U-69,593	18 ± 4	0.01
(±)-EKC	Maximal inhibition = 50%	0
(−)-EKC	>50.0	0
D-Ala^2,*N*-MePhe^4,Gly-ol^2-enkephalin	>50.0	0
D-Ala^2,D-Leu^5-enkephalin	>50.0	0
[2-D-penicillamine, 5-D-penicillamine]-enkephalin	>50.0	0
Normorphine	>50.0	0

[a] Potencies relative to TCP.

penicillamine]-enkephalin, or normoprhine. Although (±)-EKC displaced [^{3}H]TCP, its efficacy was poor and inhibition of binding plateaued at approximately 50% at 20 μM. Stereoselectivity at this site was demonstrated, in that dexoxadrol was 2-fold more potent than was levoxadrol. U-50,488H, a κ-specific ligand in guinea pig brain, exhibited high affinity (IC_{50} = 0.6 μM) for this site, but U-69,593, another κ-specific ligand, did not. A comparison of the drug potency profiles for the binding sites of the two radioligands (+)-[^{3}H]SKF-10,047 and [^{3}H]TCP revealed a poor correlation to a one-site model [Fig. 4; correlation coefficient (r) = 0.34]. However, the drug potency profiles correlate very well to a two-site model to which both ligands bind (r = 0.93 and r = 0.96 for each site). At a low concentration of [^{3}H]TCP (1 nM), σ receptor ligands displaced in a biphasic manner whereas PCP receptor ligands displaced in a monophasic manner. Collectively, these data suggest that NCB-20 cell membranes have two distinct "σ opioid" binding sites, a PCP receptor-like site and a haloperidol-sensitive σ receptor-like site. The PCP receptor-like site of NCB-20 cells, while displaying a number of pharmacological similarities to

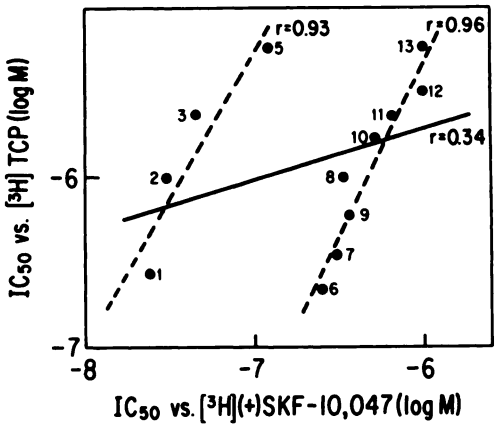

Fig. 4. Correlation of drug IC_{50} values against (+)-[^{3}H]SKF-10,047 binding sites versus [^{3}H]TCP binding sites in NCB-20 cell membranes. IC_{50} values were taken from Tables 1 and 2. Data were analyzed by linear regression analysis. The *solid line* is for a one-site model (r = 0.34). The *dashed lines* indicate two sites (r_1 = 0.93, r_2 = 0.96). *Numbers* correspond to drugs in Table 1.

that of rat brain, differs from the latter in its sensitivity to U-50,488H, (+)-3-PPP, and haloperidol as well as in its reduced separation in activity of the stereoisomers dexoxadrol and levoxadrol.

Table 3 summarizes the affinities and densities for the opioid, PCP, and σ receptors present in the NCB-20 cell line. These data suggest the presence of three classes of sites: δ opioid, haloperidol-sensitive σ, and PCP.

Discussion

This study demonstrates the presence on NCB-20 neuroblastoma hybrid cells of the δ opioid receptor, a PCP receptor, and a haloperidol-sensitive σ receptor. That the δ receptor is the only opioid (by definition, naloxone-sensitive) receptor in these cells was indicated by several findings. First, high affinity δ receptor binding was observed using the δ-specific ligand [^{3}H][2-D-penicillamine,5-D-penicillamine]-enkephalin. δ Receptor density (460 fmol/mg of protein) was approximately 3-fold greater than that observed for rat brain membranes (23). Second, the receptor density observed for [^{3}H][2-D-penicillamine,5-D-penicillamine]-enkephalin was essentially equal to that determined for the universal opioid ligand (−)-[^{3}H]bremazocine (470 fmol/mg of protein). Third, μ and κ receptor binding, measured using μ- and κ-specific labeling conditions, was undetectable. Fourth, (−)-[^{3}H]bremazocine could be totally displaced with great efficacy by the δ ligands D-Ala^2-D-Leu^5-enkephalin and [2-D-penicillamine, 5-D-penicillamine]-enkephalin.

This study represents the first demonstration of a PCP receptor-like site in a clonal cell line on the basis of *in vitro* binding. Many properties of this site are similar to those for the corresponding rat brain PCP receptor. For example, radioligand binding to the cell line receptor was of relatively high affinity, saturable, and protease- and heat-sensitive. Moreover, the rank order of potency of a series of PCP derivatives, σ opioids, and dioxalanes in the binding assay confirmed this site to be similar to the brain receptor. A few notable differences were, however, observed. For example, the affinity of [^{3}H]TCP for the cell line receptor was approximately an order of magnitude lower than that reported for the rat brain receptor (5). This finding is consistent with the recent observation (24) that the PCP receptor in cerebellum has approximately a 10-fold weaker affinity for specific ligands than that in forebrain, indicating that it may exist in different affinity states in different parts of the central nervous system. The NCB-20 cell line is derived from a neoplasm of the mouse spinal cord (25). In addition, the ligand selectivity profiles are not identical to those observed for the brain receptor. Notably, haloperidol and (+)-3-PPP have some activity at the cell line site labeled by [^{3}H]TCP, as does the κ ligand U-50,488H. This is consistent with the demonstration, in rat spinal cord membrane, of a PCP receptor that was sensitive to haloperidol (26).

As previously shown by Largent *et al.* (27), the NCB-20 cell membrane contains a haloperidol-sensitive σ receptor-like site. The binding affinity of (+)-[^{3}H]SKF-10,047 at this site (K_d = 69 nM) was found to be an order of magnitude greater than the equivalent receptor in rat brain. This binding site was demonstrated to be distinct from the PCP receptor-like site in these cells by major differences in the rank order of potency profiles. In particular, (+)-3-PPP, haloperidol, (±)-EKC, and (+)-SKF-10,047 were very efficient at inhibiting binding of (+)-[^{3}H]SKF-10,047 to the haloperidol-sensitive σ receptor-like site while much less effective at inhibiting the binding of [^{3}H]TCP to the PCP receptor-like site. Our data are consistent with labeling of both sites by (+)-[^{3}H]SKF-10,047. However, the affinity of this ligand for the PCP receptor in these cells is so low that extremely high concentrations are required, making nonspecific binding a problem. In contrast, [^{3}H]TCP appears to label both sites with equivalent affinity (Tables 1 and 2) and thus they could not be resolved in a Scatchard analysis.

The finding of a PCP receptor-like site on this cultured cell is of particular interest in that PCP receptors appear to be functionally associated with the NMDA receptor channel complex (10–12). Preliminary experiments in collaboration with D.

TABLE 3

Opioid, PCP, and σ receptors in NCB-20 cells

NCB-20 cell membranes (0.7 mg of protein/ml) were incubated with tritiated ligands indicated, as described under Materials and Methods. Receptor affinity and density values reported are means ± standard errors of averaged values from a minimum of three experiments, each performed in triplicate.

Radioligand	Receptor specificity	K_d	B_{max}
		nM	*fmol/mg of protein*
[^{3}H]D-Ala^2,*N*-MePhe^2-Gly-ol^{2}enkephalin	μ		<5
[^{3}H]U-69,593	κ		<5
[^{3}H][2-D-penicillamine,5-D-penicillamine]-enkephalin	δ	7.7 ± 0.7	460 ± 60
(−)-[^{3}H]Bremazocine	μ, δ, κ	1.3 ± 0.2	470 ± 20
[^{3}H]TCP	PCP	335 ± 56	9300 ± 1400
(+)-[^{3}H]SKF-10,047	Haloperidol-sensitive/σ	69 ± 12	4100 ± 1000

C. Spray suggested that NCB-20 cells possessed electrophysiological responses similar in pharmacological sensitivity to NMDA activated channels in neuronal tissue (28–31), although with slightly higher reversal potential (+ 10 rather than O m V). Although only a minority (less than 25%, and none of the cultures tested recently) of passages tested responded electrophysiologically to NMDA, 100% of passages tested (including some that were negative in the electrophysiological assay) exhibited [^{3}H]TCP binding. This finding indicates that PCP binding to NCB-20 cell membranes can be detected even when a functional NMDA channel cannot. It is of interest that mRNA isolated from NCB-20 cells from passages negative for the NMDA response (four of four preparations) were positive for expression of NMDA receptors in *Xenopus* oocytes; all the characteristic pharmacological features of this receptor/channel complex, including inhibition by PCP receptor ligands, were expressed.[1] This finding suggests the interesting possibility that although mRNA(s) encoding NMDA receptors are present in the NCB-20 cell, expression of the functional channel may be subject to regulation by environmental cues.

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