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Sigma-1 and Sigma-2 Receptors Are Expressed in a Wide Variety of Human and Rodent Tumor Cell Lines

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ABSTRACT

Thirteen tumor-derived cell lines of human and nonhuman origin and from various tissues were examined for the presence and density of sigma-1 and sigma-2 receptors. Sigma-1 receptors of a crude membrane fraction were labeled using [^{3}H](+)-pentazocine, and sigma-2 receptors were labeled with [^{3}H]1,3-di-*o*-tolylguanidine ([^{3}H]DTG); in the presence or absence of dextrallorphan. [^{3}H](+)-Pentazocine-binding sites were heterogeneous. In rodent cell lines (*e.g.*, C6 glioma, N1E-115 neuroblastoma, and NG108–15 neuroblastoma × glioma hybrid), human T47D breast ductal carcinoma, human NCI-H727 lung carcinoid, and human A375 melanoma, [^{3}H](+)-pentazocine bound to high- and low-affinity sites with K_d1 = 0.67–7.0 nM, B_{max}1 = 25.5–108 fmol/mg protein, K_d2 = 127–600 nM, and B_{max}2 = 942–5431 fmol/mg protein. However, [^{3}H](+)-pentazocine bound to a single site in other cell lines. In human U-138MG glioblastoma, SK-N-SH neuroblastoma, and LNCaP.FGC prostate, K_d = 28–61 nM and B_{max} = 975–1196 fmol/mg protein, whereas in ThP-1 leukemia K_d = 146 nM and B_{max} = 1411 fmol/mg protein. The sigma-1-like nature of [^{3}H](+)-pentazocine-binding sites was confirmed by competition studies which revealed high affinity for haloperidol and enantioselectivity for (+)-pentazocine over (−)-pentazocine. Interestingly, human MCF-7 breast adenocarcinoma showed little or no specific binding of [^{3}H](+)-pentazocine, suggesting the absence of sigma-1 receptors in this cell line. All cell lines examined expressed a high density of sigma-2 receptors with K_d values for [^{3}H]DTG ranging from 20 to 101 nM and B_{max} values of 491 to 7324 fmol/mg protein. Competition studies indicated possible heterogeneity of sigma-2 receptors. While sites labeled by [^{3}H]DTG in all cell lines tested exhibited affinity for haloperidol and preference for (−)-pentazocine over the (+)-enantiomer, human cell lines generally showed 4- to 7-fold lower affinity for haloperidol and approximately 10-fold lower affinity for (−)-pentazocine compared with the rodent cell lines. The high density of sigma-1 and sigma 2-binding sites in these cell lines suggests important cellular functions in cancer, as well as potential diagnostic utility for tumor-imaging agents which target sigma sites. These cell lines may be useful as model systems in which to study the functions of sigma sites in normal tissues, as well as their possible role in tumor biology.

INTRODUCTION

Because of their high affinity for most typical neuroleptic drugs, sigma receptors have received much attention as potential alternative targets for the development of antipsychotic drugs. However, their high affinity for other classes of compounds, including some anticonvulsants, antitussives, and neuroprotective agents, have suggested other possible roles of sigma sites (1–7). Details of the functional roles of sigma receptors have remained elusive, although they have been implicated in motor function, neurotransmitter synthesis and release, digestive function, regulation of smooth muscle contraction, and neurodegeneration.

Sigma receptors occur in at least two classes which are distinguishable both pharmacologically and by molecular properties (1, 8–10). The prototypic sigma ligands, haloperidol, DTG,[2] and (+)-3-PPP do not strongly differentiate the sites. However, sigma-1 sites are readily distinguished from sigma-2 sites on the basis of their affinity for benzomorphan-type opiates such as pentazocine and SKF 10,047. Sigma-1 receptors exhibit high affinity for (+)-benzomorphans and lower affinity for the corresponding (−)-enantiomer. Sigma-2 receptors show the opposite enantioselectivity, having very low affinity for (+)-benzomorphans. (−)-Benzomorphans do not readily differentiate the two sites. Other differentiating features of these two sites are as follows: (*a*) the apparent molecular weight of sigma-1 receptors is 25,000, compared to 18,000–21,500 for sigma-2, (*b*) sigma-1 receptors appear to be sensitive to guanine nucleotides, while sigma-2 receptors are not, and (*c*) sigma-1 receptors are allosterically modulated by ropizine and phenytoin, while sigma-2 sites are not. Sigma-1 receptors are selectively labeled using the (+)-benzomorphan, [^{3}H](+)-pentazocine (11). No selective probe yet exists for sigma-2 sites, but they can be labeled using [^{3}H]DTG in the presence of dextrallorphan to mask labeling of sigma-1 sites (11, 12, 13, 14).

Cell lines expressing neurotransmitter and hormone receptors have been quite useful in elucidating receptor function at the biochemical and cellular levels. Sigma receptors have previously been demonstrated in tumor-derived cell lines from rodents. Some of these include PC12 pheochromocytoma cells (8), NCB-20 cells (15, 16), and C6 glioma (13). In fact, PC12 cells, C6 glioma, and several other rodent-derived cell lines were instrumental in our initial demonstration of the existence of sigma-2 receptors (8, 13). Here we examine the existence of sigma receptor subtypes in additional cell lines of nonhuman origin, as well as in human tumor cell lines derived from various tissues and organs.

MATERIALS AND METHODS

Cell Culture. Cells were plated at a density of 2 × 10^6 cells/75-cm^2 plastic flask (Corning Co., Corning, NY) and cultured using standard procedures at 37°C in a humidified atmosphere of 5% CO_2/95% air. Human glioblastoma U-138 MG, melanoma A375 (amelanotic), neuroblastoma SK-N-SH, and breast adenocarcinoma MCF-7 were cultured in DMEM supplemented with 10% FBS. Human breast ductal carcinoma T47D, lung carcinoma NCI-H727, leukemia ThP-1, and metastatic prostate adenocarcinoma LNCaP.FGC were cultured in RPMI with 10% FBS. Rat glioma C6 and mouse neuroblastoma NB41A3 were grown in RPMI 1640 medium supplemented with 10% FBS. NG108–15 mouse neuroblastoma × rat glioma hybrid cells were grown in DMEM supplemented with 10% FBS, 1% nonessential amino acids, 1% L-glutamine, and 100 units penicillin and 100 μg streptomycin/ml medium. Mouse neuroblastomas N1E-115 and S-20Y were grown in DMEM supplemented with 10% FBS. Media were changed 3 times a week.

Membrane Preparation. Cells were allowed to grow to confluency. After decantation of the medium, the cells were washed *in situ* with HBSS. The cells were then detached by scraping with a cell scraper (Costar Corporation, Cambridge, MA) in HBSS. Suspended cells were centrifuged for 5–7 min at 1000 rpm in a cold table-top centrifuge. The cell pellet was resuspended in ice-cold 10 mM Tris-HCl, pH 7.4, containing 0.32 M sucrose to a concentration of 1 × 10^7-10^8 cells/ml and homogenized by 10–12 hand-driven strokes in a Potter-Elvehjem homogenizer (Teflon pestle). The homogenate was then cen-

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[2] The abbreviations used are: DTG, 1,3-di-*o*-tolylguanidine; (+)-3-PPP, (+)-3-(3-hydroxyphenyl)-*N*-(1-propyl)piperidine; FBS, fetal bovine serum; SKF 10,047, *N*-allylnormetazocine.

trifuged at 31,000 × g for 15 min at 4°C and the supernatant discarded. The final pellet was resuspended in ice-cold 10 mM Tris-HCl, pH 7.4, to a protein concentration of 15–20 mg/ml, and aliquots were stored at −80°C until use. Protein concentration was determined by the method of Lowry *et al.* (17) using BSA as standard.

Radioligand Binding. Sigma-1 receptors were labeled using [^{3}H](+)-pentazocine (51.7 Ci/mmol) (11). The indicated concentration of radioligand was incubated in a final volume of 0.25 ml of 50 mM Tris-HCl, pH 8.0, for 120 min at 25 or 37°C using 200 μg of membrane protein. Nonspecific binding was determined in the presence of 10 μM (+)-pentazocine (a similar level of nonspecific binding was observed using 10 μM haloperidol). Assays were terminated by addition of 5 ml of ice-cold 10 mM Tris-HCl, pH 7.4, and filtration through glass fiber filters using a Brandel (Gaithersburg, MD) cell harvester. Filters were then washed twice with 5 ml of ice-cold buffer. Filters were soaked in 0.5% polyethyleneimine for at least 30 min at 25°C prior to use. The filters were counted in CytoScint (ICN, Costa Mesa, CA) after an overnight extraction of counts. Sigma-2 receptors were labeled using [^{3}H]DTG (39.1 Ci/mmol) in the presence of 1 μM dextrallorphan (11, 13). Nonspecific binding was determined in the presence of 10 μM haloperidol. Other manipulations were as described above.

Chemicals and Cell Lines. The NG108–15 neuroblastoma × glioma hybrid cell line was the gift of Dr. R. J. Weber (National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD). N1E-115 neuroblastoma cells were obtained from Dr. Elliott Richelson (Mayo Clinic, Jacksonville, FL). Neuroblastoma S-20Y was obtained from Dr. Enrique Silva (Beth Israel Hospital, Boston, MA). NCI-H727 lung carcinoid was the gift of Dr. Terry Moody (National Cancer Institute, Bethesda, MD). All other cell lines were purchased from American Type Culture Collection (Rockville, MD).

[^{3}H](+)-Pentazocine (51.7 Ci/mmol) was synthesized by B. de Costa (National Institute of Diabetes and Digestive and Kidney Diseases), as described previously (11). [^{3}H]DTG (39.1 Ci/mmol) was purchased from DuPont/New England Nuclear (Boston, MA). Tris-HCl, polyethyleneimine, haloperidol, and tissue culture reagents (RPMI 1640, HBSS) were purchased from Sigma Chemical Company (St. Louis, MO). DMEM was purchased from GIBCO BRL (Grand Island, NY). FBS was purchased from Advanced Biotechnologies, Inc. (Columbia, MD). Enantiomers of pentazocine and SKF 10,047 were provided by Dr. Kenner C. Rice (National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD). DTG was purchased from Aldrich Chemical Company (Milwaukee, WI). (+)-3-PPP was purchased from Research Biochemicals Inc. (Natick, MA).

RESULTS

Tables 1 and 2 show the results of Scatchard analysis of [^{3}H](+)-pentazocine binding to sigma-1 sites and [^{3}H]DTG binding (in the presence or absence of dextrallorphan) to sigma-2 sites in several tumor cell lines of human and rodent origin. All cell lines examined were found to express a high density of sigma-2 receptors labeled by [^{3}H]DTG. In each case, plots were linear, suggesting labeling of a single population of sites. The K_d values for [^{3}H]DTG were generally similar across cell lines (20–46 nM) and were close to those obtained for sigma-2 sites in PC12 cells, rat liver, and rat kidney (8, 14), although the K_d value in C6 glioma, NB41A3 neuroblastoma, NG108–15 hybrid, and human U-138MG glioblastoma were somewhat higher (62–101 nM) than in the other cell lines. B_{max} values ranged from 491 fmol/mg protein in ThP-1 leukemia to 7324 fmol/mg protein in NB41A3 neuroblastoma.

Cells also expressed binding sites for [^{3}H](+)-pentazocine. However, unlike the single site for [^{3}H]DTG binding, human T47D breast ductal carcinoma, NCI-H727 lung carcinoid, A375 melanoma, and all rodent cell lines tested exhibited two apparent binding sites for [^{3}H](+)-pentazocine. In these cells, the [^{3}H](+)-pentazocine Scatchard plots were markedly curvilinear. The K_d of the high-affinity site ranged from 0.67–7.0 nM, whereas the low-affinity site had K_d values of 127–360 nM, with NG108–15 showing a low-affinity site of K_d 600 nM. In all cases in which two [^{3}H](+)-pentazocine-binding sites were apparent, the lower affinity site was present in much higher abundance compared to the higher affinity site, ranging from 16-fold higher in human T47D breast ductal carcinoma to 95-fold higher in human A375 amelanotic melanoma. However, in most cases the total density of sigma-1 binding sites (high-affinity B_{max} + low-affinity B_{max}) was comparable to the B_{max} of sigma-2 sites.

There were exceptions to the two-site labeling of [^{3}H](+)-pentazocine. MCF-7 breast adenocarcinoma exhibited little or no significant specific binding of [^{3}H](+)-pentazocine, indicating the absence of sigma-1 receptors. ThP-1 leukemia, U-138MG glioblastoma, SK-N-SH neuroblastoma, and LNCaP.FGC prostate each exhibited a high

Table 1 *Binding parameters of sigma-1 and sigma-2 receptors in membranes of various human tumor cell lines*

Sigma-1 and sigma-2 binding assays were carried out under the conditions described in "Materials and Methods" at 37°C. [^{3}H](+)-Pentazocine concentration was varied in 15 concentrations over a range of 0.1–500 nM. [^{3}H]DTG (in the presence of 1 μM dextrallorphan) was incubated in 15 concentrations at 1–400 nM. A combination of labeled and unlabeled ligand was used to achieve concentrations >50 nM for [^{3}H](+)-pentazocine and >15 nM for [^{3}H]DTG. Data were analyzed using the iterative curve-fitting program BDATA (EMF Software, Baltimore, MD). Values are the averages of 2–4 experiments ± SEM. Each experiment was carried out in duplicate.

Cell line	Sigma-1 ([^{3}H](+)-pentazocine)	Sigma-2 ([^{3}H]DTG + dextrallorphan)
MCF-7 breast adenocarcinoma	No specific binding	K_d = 24.54 ± 5.57 B_{max} = 2071 ± 734
T47D breast ductal carcinoma	K_d1 = 6.62 ± 1.03 $B_{max}1$ = 108 ± 64.6 K_d2 = 261 ± 41.48 $B_{max}2$ = 1690 ± 164	K_d = 19.95 ± 3.53 B_{max} = 1221 ± 264
NCI-H727 lung carcinoid	K_d1 = 3.81 ± 1.80 $B_{max}1$ = 25.51 ± 7.46 K_d2 = 127 ± 8.15 $B_{max}2$ = 2099 ± 313	K_d = 44.44 ± 1.78 B_{max} = 2835 ± 467
A375 melanoma (amelanotic)	K_d1 = 0.67 ± 0.23 $B_{max}1$ = 33.99 ± 12.77 K_d2 = 129 ± 12.05 $B_{max}2$ = 3223 ± 339	K_d = 34.19 ± 2.18 B_{max} = 3403 ± 348
ThP-1 leukemia	K_d = 146 ± 52.59 B_{max} = 1411 ± 102	K_d = 39.5 ± 6.72 B_{max} = 491 ± 22
U-138MG glioblastoma	K_d = 60.88 ± 13.21 B_{max} = 1115 ± 244	K_d = 80.83 ± 5.85 B_{max} = 3136 ± 871
SK-N-SH neuroblastoma	K_d = 27.99 ± 5.78 B_{max} = 975 ± 225	K_d = 32.35 ± 3.26 B_{max} = 944 ± 104
LNCaP.FGC prostate	K_d = 38.44 ± 17.78 B_{max} = 1196 ± 490	K_d = 39.00 ± 0.40 B_{max} = 727 ± 5.67

Table 2 *Binding parameters of sigma-1 and sigma-2 receptors in membranes of various rodent tumor cell lines*

Membranes were incubated using the conditions described in "Materials and Methods" and caption to Table 1, at 25°C ([^{3}H](+)-pentazocine, 13–17 concentrations; [^{3}H]DTG, 14 concentrations). Data were analyzed using the iterative curve-fitting program BDATA (EMF Software, Baltimore, MD). Values for [^{3}H](+)-pentazocine are the averages of 3–5 experiments ± SEM, each carried out in duplicate. Data for [^{3}H]DTG were taken from Vilner and Bowen (13) and are the averages of 3–5 experiments ± SEM, each carried out in duplicate. [^{3}H]DTG was incubated in the absence of dextrallorphan in these experiments. However, as shown in Table 4 and discussed in the text, 1 μM dextrallorphan had no effect on the level of specific [^{3}H]DTG binding, and [^{3}H]DTG labeled sites with a clear sigma-2 profile in rodent-derived cell lines in the absence of dextrallorphan.

Cell line	Sigma-1 ([^{3}H](+)-Pentazocine)	Sigma-2 ([^{3}H]DTG)
C6 glioma (rat)	K_d1 = 5.7 ± 1.3 $B_{max}1$ = 42.0 ± 17.4 K_d2 = 287 ± 65 $B_{max}2$ = 942 ± 136	K_d = 101 ± 7.0 B_{max} = 5507 ± 537
NB41A3 neuroblastoma (mouse)	K_d1 = 3.0 ± 1.4 $B_{max}1$ = 76.5 ± 15.2 K_d2 = 327 ± 92 $B_{max}2$ = 5431 ± 17	K_d = 62.3 ± 6.4 B_{max} = 7324 ± 670
N1E-115 neuroblastoma (mouse)	K_d1 = 3.0 ± 0.3 $B_{max}1$ = 41.5 ± 22.3 K_d2 = 360 ± 145 $B_{max}2$ = 1678 ± 134	K_d = 25.5 ± 0.4 B_{max} = 3344 ± 301
NG108-15 mouse neuroblastoma × rat glioma	K_d1 = 7.0 ± 1.2 $B_{max}1$ = 94.8 ± 1.6 K_d2 = 600 ± 197 $B_{max}2$ = 4553 ± 1687	K_d = 75.0 ± 0.7 B_{max} = 3134 ± 229
S-20Y neuroblastoma (mouse)	Not done	K_d = 46.0 ± 4.9 B_{max} = 2456 ± 135

density of an apparent single population of [^{3}H](+)-pentazocine-binding sites. Based on the K_d values, the site in ThP-1 leukemia appeared to correspond to the lower affinity [^{3}H](+)-pentazocine-binding site in cell lines such as C6 glioma and NCI-H727 lung carcinoid. Interestingly, the [^{3}H](+)-pentazocine-binding site expressed in human U-138MG glioblastoma, SK-N-SH neuroblastoma, and LNCaP.FGC prostate had K_d values intermediate between the high- and low-affinity sites and, thus, may represent an additional class of binding site.

In order to verify that [^{3}H](+)-pentazocine and [^{3}H]DTG labeled sigma-1 and sigma-2 sites as characterized in other tissues, pharmacological profiles were determined in selected cell lines. The results are shown in Table 3 for sigma-1 and Table 4 for sigma-2. Sites labeled by [^{3}H](+)-pentazocine in C6 glioma cells exhibited the pharmacological profile characteristic of sigma-1 sites, with high affinity for haloperidol, DTG, and (+)-3-PPP and enantioselectivity for (+)-benzomorphans over the corresponding (−)-enantiomer. The same was true for ductal breast, leukemia, and glioblastoma, for which the rank order of potency was: haloperidol > (+)-pentazocine > (−)-pentazocine. The competition curves for all ligands were monophasic. This indicated, that despite the presence of multiple sites as demonstrated by saturation analysis in ductal breast and C6 glioma, under the conditions of the competition assay, [^{3}H](+)-pentazocine predominantly labeled a single site with the properties of sigma-1 receptors. Furthermore, leukemia and glioblastoma, which showed only low- and moderate-affinity [^{3}H](+)-pentazocine-binding sites, respectively, also showed sigma-1-like profiles. This strongly suggests that these lower affinity sites are in fact sigma-1 receptors.

Table 4 shows that sites labeled by [^{3}H]DTG in all cell lines exhibited a sigma-2-like profile, as indicated by the high affinity for DTG, low affinity for (+)-pentazocine, and by enantioselectivity for (−)-benzomorphans over the corresponding (+)-isomer. However, although [^{3}H]DTG labeled an apparent single population of binding sites in each individual cell line (as indicated by linear Scatchard plots), a comparison of pharmacological profiles across cell lines revealed evidence for sigma-2 heterogeneity. All cell lines examined exhibited similar high affinity for DTG, moderate affinity for (+)-3-PPP and fluphenazine, and low affinity for (+)-pentazocine. However, sigma-2-like sites of the human cell lines generally exhibited about 4- to 7-fold lower affinity for haloperidol and about 10-fold lower affinity for (−)-pentazocine compared to the sigma-2 sites of the rodent cell lines. Furthermore, although human cell line [^{3}H]DTG sites exhibited the characteristic preference for (−)-pentazocine over (+)-pentazocine, the degree of enantioselectivity was much reduced

Table 3 *Pharmacological profile of sites labeled by [^{3}H](+)-pentazocine in representative cell lines*

Membranes were incubated with test ligand and 30 nM [^{3}H](+)-pentazocine in 50 mM Tris-HCl, pH 8.0, for 120 min at 37°C using the procedures described in "Materials and Methods." The high concentration of [^{3}H](+)-pentazocine was used to assure a high level of specific binding because of the low number of high-affinity sites (see Tables 1 and 2). Twelve concentrations of test ligand were used ranging from 0.05–10,000 nM or 0.5–100,000 nM. Data were analyzed using the iterative curve-fitting program GraphPAD InPlot (San Diego, CA) or CDATA (EMF Software, Baltimore, MD). Data were best fit to a one-site model. The K_i values were calculated from IC_{50} values using the Cheng-Prusoff equation (18) and K_d values shown in Tables 1 and 2. In cell lines in which two [^{3}H](+)-pentazocine-binding sites were present (C6 glioma and T47D breast ductal carcinoma), the K_d of the higher affinity site was used. This was justified since the (+)-pentazocine IC_{50} approximated the higher affinity K_d, and the competition curves had Hill coefficients near unity. Values are the averages of 2–3 experiments ± SEM. Each experiment was carried out in duplicate.

	K_i *vs.* [^{3}H](+)-pentazocine (nM)			
Ligand	C6 Glioma	T47D Breast	ThP-1 Leukemia	U-138MG Glioblastoma
Haloperidol	1.32 ± 0.29	2.18 ± 0.70	9.32 ± 0.46	52.05 ± 5.11
DTG	62.60 ± 4.12	ND[a]	ND	ND
(+)-3-PPP	114 ± 43	ND	ND	ND
Fluphenazine	175 ± 34	ND	ND	ND
(+)-Pentazocine	6.66 ± 0.54	8.80 ± 1.43	53.16 ± 9.48	302 ± 19
(−)-Pentazocine	37.31 ± 1.56	1070 ± 297	968 ± 85	5692 ± 352
(+)-SKF 10,047	409 ± 11	ND	ND	ND
(−)-SKF 10,047	2811 ± 246	ND	ND	ND

[a] ND, not done.

Table 4 *Pharmacological profile of sites labeled by [^{3}H]DTG in representative cell lines*

Competition assays were carried out and data analyzed as described in the caption to Table 3. [^{3}H]DTG was used at a concentration of 5 nM alone or 10 nM in the presence of 1 μM dextrallorphan. Results are the averages of 3–6 experiments (rodent cell lines) or 2–3 experiments (human cell lines) ± SEM. Each experiment was carried out in duplicate. Data for C6 glioma, NB41A3 neuroblastoma, and NG108-15 hybrid were taken from Vilner and Bowen (13) and were determined without the presence of dextrallorphan. The data were not affected by the absence or presence of dextrallorphan, since experiments carried out with C6 glioma, T47D breast carcinoma, and SK-N-SH neuroblastoma in the presence and absence of 1 μM dextrallorphan revealed no masking of [^{3}H]DTG binding and maintenance of the differences between human and rodent cell lines (see text). Compounds producing negligible inhibition at 10,000 nM as determined in C6 glioma were: apomorphine, (+)-10,11-dihydro-5-methyl-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801), naloxone, naltrexone, D-Ala^2,*N*-MePhe^4,Gly^5-ol-enkephalin, D-Ser^2,Leu^5,Thr^6-enkephalin, morphine, γ-amino butyric acid, and glutamate; atropine produced about 50% inhibition at 10,000 nM.

	K_i *vs.* [^{3}H]DTG (nM)							
Ligand	C6 Glioma	NB41A3 Neuroblastoma	NG108-15 Hybrid	MCF-7 Breast	T47D Breast	ThP-1 Leukemia	U-138MG Glioblastoma	SK-N-SH Neuroblastoma
Haloperidol	39.3 ± 12.8	88.8 ± 6.5	50.0 ± 12.6	212 ± 20	262 ± 37	261 ± 4.3	346 ± 72	369 ± 57
DTG	37.5 ± 11.0	49.3 ± 8.0	38.6 ± 3.1	32.1 ± 5.3	43.6 ± 3.3	ND[a]	ND	58.4 ± 8.5
(+)-3-PPP	286 ± 25	375 ± 40	493 ± 70	645 ± 71	552 ± 52	ND	ND	1469 ± 186
Fluphenazine	294 ± 28	527 ± 127	457 ± 73	791 ± 124	701 ± 22	ND	ND	954 ± 104
(+)-Pentazocine	1723 ± 33	2431 ± 323	3343 ± 1161	3172 ± 40	3739 ± 552	2116 ± 263	7684 ± 1753	9447 ± 2900
(−)-Pentazocine	320 ± 20	162 ± 29	42.5 ± 1.7	1291 ± 469	655 ± 76	1192 ± 113	3970 ± 2108	2225 ± 63
(+)-SKF 10,047	62,882 ± 7225	91,822 ± 4190	19,337 ± 6844	37,302 ± 5861	ND	ND	ND	39,740 ± 4329
(−)-SKF 10,047	6122 ± 529	4074 ± 340	3513 ± 1316	12,059 ± 1894	ND	ND	ND	41,461 ± 5801

[a] ND, not done.

compared to the rodent lines because of the lower affinity for (−)-pentazocine. Similar observations can be made for the enantiomers of SKF 10,047 when rodent and human cell lines are compared.

We investigated the possibility that the differences between the human cell lines and rodent cell lines could be due in some way to the fact that assays with the rodent lines were carried out without 1 μM dextrallorphan present, whereas all assays with human cell lines were carried out in the presence of 1 μM dextrallorphan. Since possible interference from sigma-1 receptors might be different in cell lines having different complements of [^{3}H](+)-pentazocine-binding sites, a comparison was done using C6 glioma, T47D breast carcinoma, and SK-N-SH neuroblastoma. These cell lines were selected since they represent rat and human cell lines which have high- and low-affinity [^{3}H](+)-pentazocine-binding sites (C6 glioma and T47D breast carcinoma) and a human line (SK-N-SH neuroblastoma) with a single, moderate-affinity [^{3}H](+)-pentazocine-binding site. Haloperidol, (−)-pentazocine, and DTG were competed versus 10 nM [^{3}H]DTG in the absence and presence of 1 μM dextrallorphan. First, in each individual cell line, there was no difference in the level of specific [^{3}H]DTG binding in the absence and presence of 1 μM dextrallorphan. This shows that [^{3}H]DTG produced no detectable sigma-1 receptor labeling under these conditions, presumably because of the very high density of sigma-2 sites relative to high-affinity sigma-1 sites. Also, in each cell line, haloperidol, (−)-pentazocine, and DTG had the same K_i values whether or not 1 μM dextrallorphan was present. Importantly, the differences between C6 glioma and the two human cell lines were preserved, whether or not dextrallorphan was present, with haloperidol and (−)-pentazocine having considerably lower affinity in the human lines and DTG having the same affinity in all three cell lines. Thus, the apparent sigma-2 heterogeneity indicated by the subtle differences in pharmacological profile is not due to the method of assay.

DISCUSSION

The data presented here show that tumor cell lines of various tissue origin and species express sigma-1 or sigma-2 receptors in high density. Most cell lines examined expressed both subtypes. However, it is quite noteworthy that MCF-7 breast adenocarcinoma failed to exhibit any specific [^{3}H](+)-pentazocine binding and, as such, is the first cell or tissue examined to date which does not possess sigma-1 receptors. This cell line should be useful in functional studies of sigma-2 receptors, since ligands selective for the sigma-2 subtype over sigma-1 are not yet widely available.

Interestingly, both [^{3}H](+)-pentazocine- and [^{3}H]DTG-binding sites were heterogeneous. [^{3}H](+)-Pentazocine labeled high- and low-affinity sites in most of the cell lines examined. This was not observed in any other tissues examined (11, 14). The high-affinity site (K_d 1–7 nM) had a K_d value similar to sigma-1 sites found in other tissues (11, 14). The lower affinity site (K_d 125–360 nM) was much more abundant, with B_{max} values 16- to 95-fold higher than that of the high-affinity site. One possible explanation for the presence of high- and low-affinity [^{3}H](+)-pentazocine-binding sites is that the lower affinity site represents labeling of sigma-2 receptors, which occur in high abundance in the cell lines. However, the K_d values in the 125–360 nM range would be too low for sigma-2 sites, since (+)-pentazocine exhibits sigma-2 binding constants in the micromolar range (8, 11, 14). In addition, linear plots are observed with [^{3}H](+)-pentazocine in rat liver and kidney membranes, where the density of sigma-2 sites is 3-fold higher than sigma-1 (14). Moreover, the human leukemia cell line expressed a single [^{3}H](+)-pentazocine-binding site with a K_d value (146 nM) comparable to the lower affinity site in cells which expressed two sites. This may suggest that the low- and high-affinity sites are distinct entities which can exist in different cells and do not represent interconvertible receptor states. Also, guanine nucleotides [GTP and Gpp(NH)p] at concentrations up to 1 mM had no significant effect on [^{3}H](+)-pentazocine binding to C6 glioma cell membranes (not shown), suggesting that the high- and low-affinity sites on these cells are not the result of receptor interactions with G-proteins. Finally, the intermediate affinity (K_d 30–60 nM) of sites in human neuroblastoma, glioblastoma, and prostate cell lines may suggest yet a third type of [^{3}H](+)-pentazocine-binding site.

Despite the apparent heterogeneity indicated by saturation analysis, competition analysis in four cell lines representing cells with the various complements of [^{3}H](+)-pentazocine-binding sites (both high and low affinity, low affinity only, and intermediate affinity) revealed a sigma-1-like profile in each (Table 3). Based on the current data, it cannot be said with certainty that the [^{3}H](+)-pentazocine-binding sites having different K_d values represent different subclasses of sigma-1 receptor, since other factors could account for this. The nature of the apparent heterogeneity of sigma-1 receptors or [^{3}H](+)-pentazocine-binding sites is currently under more detailed investigation.

Sigma-2 sites also appeared to show heterogeneity. The pharmacological profile observed in the rodent-derived cell lines was similar to sigma-2 receptors found in other tissues (11, 14). Whereas all cell lines showed similar affinity for DTG, (+)-3-PPP, fluphenazine, and (+)-pentazocine, the human-derived lines showed a significantly lower affinity for haloperidol and (−)-pentazocine compared to the rodent cell lines. Also, the human cell lines were characterized by a

lower difference in affinity between the (+)- and (−)-enantiomers of benzomorphans. The lower affinity of human lines for haloperidol is particularly significant, since high haloperidol affinity (<50 nM) is a hallmark of both sigma-1 and sigma-2 receptors (1, 10).

The apparent sigma-2 heterogeneity was not due to the method of assay, since similar results were obtained in the absence and presence of 1 μM dextrallorphan when a direct comparison was made in C6 glioma, T47D breast carcinoma, and SK-N-SH neuroblastoma using 10 nM [^{3}H]DTG. Furthermore, the sigma-2 heterogeneity does not appear to be related to the types of [^{3}H](+)-pentazocine-binding sites present in the cells. For example, C6 glioma and T47D breast carcinoma have the same complement of [^{3}H](+)-pentazocine sites and yet show the differences in profile, whereas T47D breast carcinoma, SK-N-SH neuroblastoma, and ThP-1 leukemia all have different complements of [^{3}H](+)-pentazocine-binding sites and yet show similar profiles. In addition, MCF-7 breast cells show the profile difference when compared to the rodent cell lines, despite the fact that these cells do not possess any specific [^{3}H](+)-pentazocine-binding activity.

The sigma-2-like site of the human cell lines shares some, but not all, characteristics with a "low-affinity" sigma-like site previously described in NCB-20 cells (16). This site exhibited affinity for haloperidol and a slight preference for (−)-benzomorphans over (+)-benzomorphans, suggesting a relationship with the sigma-2 receptors found in other tissues. However, the lower affinity for haloperidol (K_i 508 nM), (−)-pentazocine (K_i 1532 nM), (+)-3-PPP (K_i 8246 nM), and several other sigma ligands demonstrated this site to be distinct. It is not clear whether the apparent sigma-2 heterogeneity among cell lines described in the present study represents species differences, sigma-2 receptor subtypes, or some other phenomenon. This will require further study using additional tissues and ligands, particularly neuroleptics and enantiomeric benzomorphans.

Among the cell lines examined in the current study, the number of sigma receptors expressed per cell is very high. For example, C6 glioma, NG108–15 neuroblastoma-glioma hybrid, and N1E-115 neuroblastoma express about 0.3, 1.3, and 1.4 million sigma-2 receptors per cell, respectively. It is likely that this level of expression is quite different from that of normal tissue. The B_{max} values observed in membranes from normal tissues such as brain, liver, and kidney (8, 11, 14) represent the density of sites in a heterogeneous cell population, some of which may have high and some of which may have low sigma receptor density. As such, the B_{max} values from normal tissues represent an average receptor density, and it is therefore not possible to accurately estimate the density on any given cell type within the tissue. However, when this is taken into account, high-affinity sigma-1 sites would occur in much lower density, whereas sigma-2 sites would occur in much higher density in these cell lines compared to normal tissues. This would be consistent with the results of others who showed that sigma receptors labeled with [^{3}H]DTG are overexpressed in human solid tumors from various body sites relative to surrounding normal tissue (19, 20). The high level of sigma receptor expression in tumor-derived cell lines and solid tumors suggests the likelihood that these receptors subserve an important function in cancer cells.

There are other implications of the high sigma receptor density for tumor biology. We have shown that sigma ligands produce marked changes in the morphology and viability of tumor-derived cell lines with potencies which approximately parallel their rank order of affinity at sigma receptors (21–23). The neuroleptics haloperidol, reduced haloperidol, and fluphenazine as well as the novel sigma ligands BD737, BD1008, SH344, and JL-II-147 produced morphology changes and cytotoxic effects in several rodent cell lines and also in the human neuroblastomas SK-N-SH and SH-SY5Y (23). Cytotoxic effects were also seen in U-138MG glioblastoma, MCF-7 breast adenocarcinoma, and A375 melanoma.[3] These results suggest that sigma receptors play some important role in the maintenance of cellular viability and the possible utility of sigma ligands as antitumor agents. In addition, we have shown that the novel iodinated probes *N*-[2-(piperidinylamino)ethyl]-4-iodobenzamide (IPAB) and (*N*-benzylpiperidin-4-yl)-4-iodobenzamide (4-IBP) bind with high affinity and selectivity to sigma sites of guinea pig brain or rat liver, and the ^{125}I-labeled derivatives bound with nanomolar affinity to sigma sites of human melanoma and MCF-7 breast carcinoma cells, respectively (24, 25). Furthermore, [^{131}I]PAB produced clear scintigraphic images of the tumor in nude mice with human malignant melanoma xenografts (24). Thus, sigma sites may be useful as markers in the noninvasive detection and visualization of a wide variety of tumors using single proton emission computed tomography and positron emission tomography technology.

We have demonstrated the presence of sigma-1 and sigma-2 receptor subtypes in several tumor cell lines from both rodent and human. Further investigation will be needed in order to elucidate the function of sigma-binding sites and their ligands in these cells. The use of cell lines expressing sigma-1 and sigma-2 receptors will also facilitate ongoing studies of sigma receptors in the brain and peripheral tissues.

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