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

Bertold J. Vilner, Christy S. John, and Wayne D. Bowen

Unit on Receptor Biochemistry and Pharmacology, Laboratory of Medicinal Chemistry, National Institute of Diabetes Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892 [B. J. V., W. D. B.], and Radiopharmaceutical Chemistry Section, Department of Radiology, The George Washington University Medical Center, Washington, DC 20037 [C. S. J.]

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 [3H](+)-pentazocine, and sigma-2 receptors were labeled with [3H]DGTG; in the presence or absence of dextrorphan. [3H](+)-Pentazocine-binding sites were heterogeneous. In rodent cell lines (e.g., C6 glioma, NIE-115 neuroblastoma, and NG108-15 neuroblastoma × glioma hybrid), human T47D breast ductal carcinoma, human NCI-H727 lung carcinoid, and human A375 melanoma, [3H](+)-pentazocine bound to high- and low-affinity sites with \( K_d = 0.67-7.0 \text{nM} \) and \( B_{max} = 25.5-105 \text{fmol/mg protein} \) for sigma-1 sites and \( K_d = 127-600 \text{nM} \) and \( B_{max} = 942-5431 \text{fmol/mg protein} \) for sigma-2 sites. However, [3H](+)-pentazocine bound to a single site in all cell lines. In human U-138MG glioblastoma, SK-N-SH neuroblastoma, and LNCaP.FGC prostate, \( K_d = 28-61 \text{nM} \) and \( B_{max} = 975-1196 \text{fmol/mg protein} \), whereas in Th-1 leukemia \( K_d = 146 \text{nM} \) and \( B_{max} = 1411 \text{fmol/mg protein} \). The sigma-1-like nature of [3H](+)-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 [3H](+)-pentazocine, suggesting the absence of sigma-1 receptors in this cell line. 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 receptors. 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 various classes of compounds, including some anticonvulsants, antitussives, and neuroprotective agents, has 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, 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 ropivaine and phenytoin, while sigma-2 sites are not. Sigma-1 receptors are selectively labeled using the (+)-benzomorphan, [3H](+)-pentazocine (11). No selective probe yet exists for sigma-2 sites, but they can be labeled using [3H]DGTG in the presence of dextrorphan 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 × 10⁶ cells/75-cm² plastic flask (Corning Co., Corning, NY) and cultured using standard procedures at 37°C in a humidified atmosphere of 5% CO₂/95% 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 carcinoid NCI-H727, leukemia Th-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 NIE-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 × 10⁶-10⁷ 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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1 To whom requests for reprints should be addressed, at Unit on Receptor Biochemistry and Pharmacology, NIDDK/Laboratory of Medicinal Chemistry, Bldg. 8, Rm. B1-23, 8 Center Dr. MSC 0815, Bethesda, MD 20092-0815.

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 pel-let 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 [3H](+)-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 counted in CytoScint (ICN, Costa Mesa, CA) after an overnight extraction of counts. Sigma-2 receptors were labeled using [3HjDTG (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).

[3H](+)-Pentazocine (51.7 Ci/mmol) was synthesized by B. de Costa (National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD). [3H]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 [3H](+)-pentazocine binding to sigma-1 sites and [3H]DTG binding (in the presence or absence of dextrallorphan) to sigma-2 sites in various 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 [3H]DTG. In each case, plots were linear, suggesting labeling of a single population of sites. The Kd values for [3H]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 Kd value in C6 glioma, NB41A3 neuroblastoma, NCI-H727—15 hybrid, and human U-138MG glioblastoma were somewhat higher (62—101 nm) than in the other cell lines. Bmax 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 [3H](+)-pentazocine. However, unlike the single site for [3H]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 [3H](+)-pentazocine. In these cells, the [3H](+)-pentazocine Scatchard plots were markedly curvilinear. The Kd of the high-affinity site ranged from 0.67—7.0 nm, whereas the low-affinity site had Kd values of 127—360 nm, with NG108—15 showing a low-affinity site of Kd 600 nm. In all cases in which two [3H](+)-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 Bmax + low-affinity Bmax) was comparable to the Bmax of sigma-2 sites.

There were exceptions to the two-site labeling of [3H](+)-pentazocine. MCF-7 breast adenocarcinoma exhibited little or no specific binding of [3H](+)-pentazocine, indicating the absence of sigma-1 receptors. ThP-1 leukemia, U-138MG glioblastoma, SK-N-SH neuroblastoma, and LNCaP.26C prostate each exhibited a high

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

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Sigma-1 <a href="+">3H</a>-pentazocine</th>
<th>Sigma-2 [3H]DTG + dextrallorphan</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7 breast adenocarcinoma</td>
<td>No specific binding</td>
<td>Kd = 24.54 ± 5.57 Bmax = 2071 ± 734</td>
</tr>
<tr>
<td>T47D breast ductal carcinoma</td>
<td>Kd = 6.62 ± 1.03 Bmax = 108 ± 64.6</td>
<td>Kd = 19.95 ± 3.53 Bmax = 1221 ± 264</td>
</tr>
<tr>
<td>NCI-H727 lung carcinoid</td>
<td>Kd = 3.81 ± 1.80 Bmax = 25.21 ± 7.46</td>
<td>Kd = 26.85 ± 4.67</td>
</tr>
<tr>
<td>A375 melanoma (amelanotic)</td>
<td>Kd = 0.67 ± 0.23 Bmax = 33.99 ± 12.77</td>
<td>Kd = 34.19 ± 2.18 Bmax = 3403 ± 348</td>
</tr>
<tr>
<td>ThP-1 leukemia</td>
<td>Kd = 146 ± 52.59 Bmax = 1411 ± 102</td>
<td>Kd = 39.5 ± 6.72 Bmax = 491 ± 22</td>
</tr>
<tr>
<td>U-138MG glioblastoma</td>
<td>Kd = 60.88 ± 13.21 Bmax = 1115 ± 244</td>
<td>Kd = 80.83 ± 5.85 Bmax = 3136 ± 871</td>
</tr>
<tr>
<td>SK-N-SH neuroblastoma</td>
<td>Kd = 27.99 ± 5.78 Bmax = 975 ± 225</td>
<td>Kd = 32.35 ± 3.26 Bmax = 944 ± 104</td>
</tr>
<tr>
<td>LNCaP.26C prostate</td>
<td>Kd = 38.44 ± 17.78 Bmax = 1196 ± 490</td>
<td>Kd = 39.00 ± 0.40 Bmax = 727 ± 5.67</td>
</tr>
</tbody>
</table>
density of an apparent single population of [3H](+)-pentazocine-binding sites. Based on the Kd values, the site in ThP-1 leukemia appeared to correspond to the lower affinity [3H](+)-pentazocine-binding site in cell lines such as C6 glioma and NCI-H727 lung carcinoid. Interestingly, the [3H](+)-pentazocine-binding site expressed in human U-138MG glioblastoma, SK-N-SH neuroblastoma, and LNCaP.FGC prostate had Kd values intermediate between the high- and low-affinity sites and, thus, may represent an additional class of binding site.

In order to verify that [3H](+)-pentazocine and [3H]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 [3H](+)-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 (−)-isomer. However, although [3H]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 [3H]DTG sites exhibited the characteristic preference for (+)-pentazocine over (+)-pentazocine, the degree of enantioselectivity was much reduced.
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, 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 [3H](+)-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 [3H](+)-pentazocine-binding sites (C6 glioma and T47D breast carcinoma) and a human line (SK-N-SH neuroblastoma) with a single, moderate-affinity [3H](+)-pentazocine-binding site. Haloperidol, (−)-pentazocine, and DTG were competed versus 10 nM [3H]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 [3H]DTG binding in the absence and presence of 1 μM dextrallorphan. This shows that [3H]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 Ki 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 [3H](+)-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 [3H](+)-pentazocine- and [3H]DTG-binding sites were heterogeneous. [3H](+)-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_i 1–7 nM) had a K_a value similar to sigma-1 sites found in other tissues (11, 14). The lower affinity site (K_a 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 [3H](+)-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_a 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 [3H](+)-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 [3H](+)-pentazocine-binding site with a K_a 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 [3H](+)-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_a 30–60 nM) of sites in human neuroblastoma, glioblastoma, and prostate cell lines may suggest yet a third type of [3H](+)-pentazocine-binding site.

Despite the apparent heterogeneity indicated by saturation analysis, competition analysis in four cell lines representing cells with the various complements of [3H](+)-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 [3H](+)-pentazocine-binding sites having different K_a 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 [3H](+)-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 benzomorphan. 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 dextralorphan when a direct comparison was made in C6 glioma, T47D breast carcinoma, and SK-N-SH neuroblastosoma using 10 nm [3H]DTG. Furthermore, the sigma-2 heterogeneity does not appear to be related to the types of [3H](+)-pentazocine-binding sites present in the cells. For example, C6 glioma and T47D breast carcinoma have the same complement of [3H](+)-pentazocine sites and yet show the differences in profile, whereas T47D breast carcinoma, SK-N-SH neuroblastosoma, and ThPB-1 leukemia have different complements of [3H](+)-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 [3H](+)-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 (Ki 508 nm), (−)-pentazocine (K 1532 nm), (+)-3-PPP (K 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 neuroblastosoma-glioma hybrid, and N1E-115 neuroblastosoma 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 Bmax 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 Bmax 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 [3H]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-11-147 produced morphological 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. These results suggest that sigma receptors play an 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 that the 123I-labeled derivatives bound with nanomolar affinity to sigma sites of human melanoma and MCF-7 breast carcinoma cells, respectively (24, 25). Furthermore, [131I]IPAB 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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