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Cytotoxicity of σ-Receptor Ligands Is Associated with Major Changes of Cellular Metabolism and Complete Occupancy of the σ-2 Subpopulation

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Tumor cells can be selectively killed by application of σ-ligands; high concentrations (20–100 μM) are often required, however, because either diffusion barriers must be passed to reach intracellular sites or the entire σ-receptor population should be occupied to induce cell death. We measured receptor occupancies associated with the cytotoxic effect and dose-dependent changes of cellular metabolism in a tumor cell line. **Methods:** C6 cells (rat glioma) were grown in monolayers and exposed to (+)-pentazocine (σ-1 agonist), AC915 (σ-1 antagonist), rimcazole (σ-1/σ-2 antagonist), or haloperidol (σ-1/σ-2 antagonist). Occupancy of σ-receptors by the test drugs was measured by studying the competition of the test drugs with cellular binding of the ligand ^{11}C-SA4503. Metabolic changes were quantified by measuring cellular uptake of ^{18}F-FDG, ^{18}F-FLT, ^{11}C-choline, or ^{11}C-methionine. Cytotoxicity was assessed by cellular morphology observation and cell counting after 24 h. **Results:** IC_{50} values (drug concentrations resulting in 50% occupancy of the available binding sites) of the test drugs for inhibition of cellular ^{11}C-SA4503 binding were 6.5, 7.4, 0.36, and 0.27 μM for (+)-pentazocine, AC915, rimcazole, and haloperidol, respectively. EC_{50} values (dose required for a 50% reduction of cell number after 24 h) were 710, 819, 31, and 58 μM, for pentazocine, AC915, rimcazole, and haloperidol, respectively. Cytotoxic doses of σ-ligands were generally associated with increased uptake of ^{18}F-FDG, decreased uptake of ^{18}F-FLT and ^{11}C-choline, and little change in ^{11}C-methionine uptake per viable cell. **Conclusion:** IC_{50} values of the test drugs reflect their in vitro affinities to σ-2 rather than to σ-1 receptors. Because cytotoxicity occurred at concentrations 2 orders of magnitude higher than IC_{50} values for inhibition of cellular ^{11}C-SA4503 binding, high (99%) occupancy of σ-2 receptors is associated with loss of cell viability. ^{18}F-FLT, ^{11}C-choline, and ^{18}F-FDG responded most strongly to drug treatment and showed changes corresponding to the cytotoxicity of the test compounds.

Key Words: PET; σ-receptor occupancy; cellular proliferation; glioma cells; ^{18}F-FLT; ^{18}F-FDG; ^{11}C-choline; ^{11}C-methionine

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Sigma receptors are unique proteins integrated in plasma and endoplasmatic reticulum membranes of tissue derived from immune, endocrine, and reproductive organs; kidney; liver; and brain (*1*). σ-receptors were initially considered as a subtype of the opioid receptor but were later shown to be unlike any other known neurotransmitter or hormone binding site (*2*). After 30 y of extensive research, understanding of the molecular cascade triggered by these transmembrane proteins is still rudimentary (*3*). The 2 confirmed σ-receptor subtypes, 25-kD σ-1 and 21.5-kD σ-2 (*4*), are strongly overexpressed in rapidly proliferating cells, for example, in cancer cells from animals and humans, as compared with healthy tissue (*5–7*). Although the sequence and partial structure of the σ-1 receptor are already established (*8,9*), the existence of the σ-2 receptor has been proven only pharmacologically (*10*).

Many natural substances from plant extracts and newly synthesized compounds were tested for σ-receptor binding, resulting in the availability of a broad range of σ-receptor ligands today (*11*). Interestingly, some of these compounds were shown to be effective antineoplastic agents (*12,13*). Later, in vitro (*14–16*) and in vivo (*17,18*) studies confirmed that σ-ligands such as haloperidol and rimcazole inhibit growth of both cultured cancer cells and in vivo tumors, whereas they do not affect proliferation or survival of the noncancer tissue (*18*). These studies highlighted the potency of σ-ligands to kill cancer cells with minimal side effects. In most cell lines, including in C6 rat glioma, cell death was observed only after administration of high concentrations (20–100 μM) of a σ-1 receptor antagonist or a σ-2 receptor agonist (*12,13*). The reason for the requirement of high concentrations is unknown. Possibly biomembranes limit drug access to intracellular sites, or a high fraction of the σ-receptor population should be occupied to kill tumor cells.

If a suitable radioligand for σ-receptors is available, dose-dependent occupancy of the receptor population by an exogenous ligand can be assessed by performing a competition assay in intact cells. Measured occupancy values can

be subsequently compared with the dose-dependent effects of test drugs on cellular morphology and growth. The σ-1 agonist SA4503, labeled with the positron emitter ^{11}C, is available for this purpose (*19*). We used C6 cells as an in vitro model for the measurement of both σ-receptor occupancy and cytotoxic effects. This rat glioma cell line has been used extensively in previous research because it expresses high densities of both σ-1 and σ-2 receptors (*6*). Rimcazole (σ-1/σ-2 receptor antagonist), haloperidol (σ-1/σ-2 receptor antagonist), (+)-pentazocine (σ-1 receptor agonist), and AC915 (σ-1 receptor antagonist) were chosen as our model compounds because these drugs are commercially available and are potent σ-receptor ligands (*20–23*). Several metabolic PET tracers, such as ^{18}F-FDG, 3′-deoxy-3′-fluorothymidine (^{18}F-FLT), ^{11}C-choline, and ^{11}C-methionine, are routinely produced in our laboratory. These radiopharmaceuticals track changes in cellular metabolism and may show altered uptake kinetics after antitumor therapy (*24*). Thus, we could assess the dose-dependent effects of σ-ligands on cellular biochemistry.

The present study aimed to answer the following questions: Which concentrations of σ-ligands should be administered to occupy a substantial fraction of the intracellular receptor population and kill C6 cells? At which level of receptor occupancy does cell death occur? Can PET tracers detect any changes of cellular metabolism after σ-ligand binding? Which PET tracer is the most sensitive indicator of reduced cellular viability?

MATERIALS AND METHODS

Culture Medium and Drugs

Dulbecco's minimum essential medium (DMEM), fetal calf serum (FCS), and trypsin were products of Invitrogen. AC915, haloperidol, (+)-pentazocine, rimcazole, and trypan blue (0.4% solution in phosphate-buffered saline) were purchased from Sigma. AC915 was dissolved in water with slight heating, and haloperidol and rimcazole were dissolved in ethanol. Stock solutions of (+)-pentazocine were made in 0.1N hydrochloric acid.

Radiopharmaceuticals

^{11}C-methionine, ^{11}C-choline, ^{18}F-FLT, and ^{18}F-FDG were prepared by standard procedures reported in the literature (*25*). ^{11}C-SA4503 was prepared by reaction of ^{11}C-methyl iodide with the appropriate 4-*O*-methyl precursor (*26*). Specific radioactivity at the end of synthesis was greater than 22 TBq/mmol. All radiochemical purities were greater than 95%.

Cell Culture

C6 rat glioma cells obtained from the American Type Culture Collection were grown as monolayers in DMEM (high glucose) supplemented with 7.5% FCS in a humidified atmosphere of 5% CO_2/95% air at 37°C. Before each experiment, the cells were seeded in 12-well plates (Costar). An equal number of cells was dispensed in each well and was supplied with 1.1 mL of DMEM (high glucose) supplemented with 7.5% FCS.

Binding Studies

Binding studies were performed 48 h after seeding cells in 12-well plates, when confluency reached 80%−90%. Various concentrations of an unlabeled competitor (AC915, haloperidol, (+)-pentazocine, or rimcazole; range, 10^{-8} to 10^{-3} M, in triplicate) were dispensed to the culture medium in the wells. After 2 min, 4 MBq of ^{11}C-SA4503 in less than 30 μL of saline (containing 30% ethanol) were added to each well. After about 45 min of incubation, the medium was quickly removed, and the monolayer was washed 3 times with phosphate-buffered saline. Cells were then treated with 0.2 mL of trypsin. When the monolayer had detached from the bottom of the well, 1 mL of DMEM (high glucose) supplemented with 7.5% FCS was applied to stop the proteolytic action. Cell aggregates were resolved by repeated (at least 10-fold) pipetting of the trypsin/DMEM mixture. Radioactivity in the cell suspension (1.2 mL) was assessed using a γ-counter (Compugamma 1282 CS; LKB-Wallac). A sample of the suspension was mixed with trypan blue solution (1:1 v/v) and used for cell counting. Cell numbers were manually determined, using a phase-contrast microscope (Olympus), a Bürker bright-line chamber (depth, 0.1 mm; 0.0025 mm^2 squares), and a hand-tally counter.

Uptake of radioactivity normalized to the number of viable cells was plotted against the logarithm of the dose of the competing drug (in [M]). A 3-parameter curve (single-site competition model) was fitted to these data, using the following equation:

$$Y = \text{bottom} + (\text{top} - \text{bottom})/(1 + 10^{(X - \log IC_{50})}),$$

where IC_{50} is drug concentration resulting in 50% occupancy of the available binding sites. GraphPad Prism (GraphPad Software) was used for curve fitting and graphical presentation of data. Less than 6% (in most wells $<<5\%$) of the administered tracer dose was bound to the cells under these conditions.

Treatment with σ-Ligands and Scoring of Morphologic Effects

σ-Ligands were administered 24 h after seeding cells in 12-well plates when confluency had reached 40%−45%. Final concentrations in culture medium were 1, 3, 10, 30, and 100 (in some cases also 300) times the IC_{50} value of the test drug for inhibition of ^{11}C-SA4503 binding to cellular σ-receptors (determined as described above).

After 20–24 h of incubation with σ-ligands, the cells were monitored by phase-contrast microscopy. The effect of each compound and concentration was scored relative to a control receiving only solvent vehicle. Scoring was performed as described previously (*12*). Experiments were performed in quadruplicate and repeated at least twice. Decreases of cell number after drug treatment were quantified by harvesting and resuspending cells in medium containing FCS, followed by manual counting as described above.

Uptake Studies

Cellular uptake of metabolic PET tracers was determined after 24 h of treatment with either vehicle or a σ-ligand. Experiments were performed in quadruplicate and repeated at least twice. At time zero, a PET tracer (4 MBq of ^{11}C-methionine or ^{11}C-choline or 2 MBq of ^{18}F-FDG or ^{18}F-FLT in <20 μL of saline) was added to each well. After 45 min of incubation, cells were washed, and cellular radioactivity was assessed as described above. Less than 1% (^{11}C-methionine), 2% (^{18}F-FDG), or 4% (^{11}C-choline and

^{18}F-FLT) of the administered tracer dose was taken up by the cells under these conditions.

Statistics

Differences between groups were examined using 1-way ANOVA. A P value of less than 0.05 was considered statistically significant.

RESULTS

Receptor Occupancy

Curves describing competition between the test drugs and the radioligand ^{11}C-SA4503 for cellular σ-receptors are presented in Figure 1. The data were best fitted by a 1-site competition model. IC_{50} values were either in the submicromolar range (rimcazole, 0.36 μM; haloperidol, 0.27 μM) or in the micromolar range ((+)-pentazocine, 6.5 μM; AC915, 7.4 μM).

Subtype Involvement

Table 1 lists literature values for the affinities (K_d) of the test compounds to σ-1 and σ-2 receptors, determined by in vitro binding assays using membrane fragments isolated from rodent tissues. We observed much higher (60- to 1,500-fold) IC_{50} values in intact cells than known K_d values at σ-1 receptors, particularly in the case of AC915, (+)-pentazocine, and haloperidol. However, IC_{50} values of all test compounds corresponded closely to their K_d values at σ-2 receptors. Here, only minor (<3-fold) differences were observed. Therefore, IC_{50} values for inhibition of ^{11}C-SA4503 binding to intact cells corresponded to the σ-2 K_d rather than to the σ-1 K_d of the test compounds.

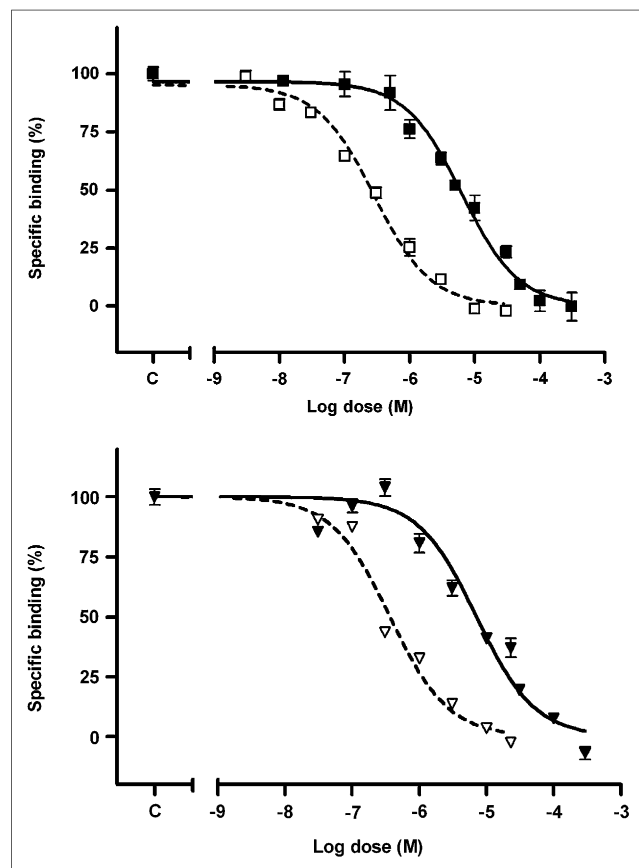

FIGURE 1. Dose-dependent increase of cellular ^{11}C-SA4503 binding by (+)-pentazocine (■), haloperidol (□), AC915 (▼), and rimcazole (▽). Error bars indicate SEM. Specific binding was calculated by subtracting nonspecific binding (i.e., residual binding of radioligand in presence of highest dose of competing drug) from total binding; it amounted to 70%−80% of total radioactivity uptake by cells.

Cytotoxicity

When the σ-antagonists rimcazole, haloperidol, or AC915 were applied to C6 cells in culture, a dose-dependent growth inhibition was observed after 24 h (Fig. 2). Low doses of the test drugs did not affect cellular growth. Above a certain threshold concentration (rimcazole, 20 μM; haloperidol, 25 μM; and AC915, 220 μM), a reduction in cell number became apparent. EC_{50} values of the test compounds (effective concentrations resulting in 50% cell loss) were somewhat higher than the threshold for cytotoxicity (rimcazole, 31 μM; haloperidol, 58 μM; and AC915, 819 μM). Virtually complete cell death was observed after 24 h of treatment with either 100 μM rimcazole or 150 μM haloperidol (Fig. 2). Even 200 μM σ-1 agonist (+)-pentazocine did not yet affect cell division or viability. Here, the EC_{50} value for growth inhibition was 710 μM.

Receptor Occupancy at Cytotoxic Dose

By comparing EC_{50} values of the test compounds with their IC_{50} values for inhibition of ^{11}C-SA4503 binding in intact cells, σ-receptor occupancies at cytotoxic doses of the test drugs could be estimated, using the curves presented in Figure 1 or the formula for a single-site competition model (see "Materials and Methods"). Because EC_{50} values were about 2 orders of magnitude higher than the corresponding IC_{50} values of the test drugs, cell death appeared to be associated with virtually complete (i.e., 99%) occupancy of the σ-receptor population (Table 2).

Changes of Cellular Metabolism

The metabolic PET tracers that we used responded differently to treatment of C6 cells with σ-receptor ligands. Cytotoxic doses of (+)-pentazocine, haloperidol, and rimcazole were generally associated with increases of the uptake of ^{18}F-FDG, decreases of the uptake of ^{18}F-FLT and ^{11}C-choline, and little change of ^{11}C-methionine uptake per viable cell (Figs. 3–6).

Significant increases of ^{18}F-FDG uptake were observed only when a certain threshold concentration was exceeded. The threshold dose for haloperidol and rimcazole was 10 μM but in the case of (+)-pentazocine was 30 μM (Fig. 3). Maximal increases of ^{18}F-FDG uptake (+111%, +150%, and +166% for haloperidol, rimcazole, and (+)-pentazocine, respectively; all statistically significant) occurred at doses of 100 μM for haloperidol, 112 μM for rimcazole, and 660 μM for (+)-pentazocine. Higher doses of test drug could

TABLE 1
Binding of Test Compounds to σ-Receptor Subtypes

Test drug	K_d (nM)		IC_{50} for inhibition of ^{11}C-SA4503 binding in C6 cells (nM)	IC_{50}/K_d	
	σ_1-receptor	σ_2-receptor		σ_1-receptor	σ_2-receptor
AC915	4.9 (rat liver) (*22*)	>10,000 (rat liver) (*22*)	7,358	1,502	0.7
(+)-Pentazocine	7.0 (rat brain) (*21*); 5.5 (rat brain) (*23*)	2,470 (rat liver) (*23*); 1,923 (rat liver) (*38*)	6,495	928–1,181	2.6–3.4
Haloperidol	4.7 (rat brain) (*39*); 2.6 (rat brain) (*23*)	167 (rat liver) (*23*)	274	58–105	1.6
Rimcazole	690 (guinea pig brain) (*20*)	180 (guinea pig brain) (*20*)	356	0.5–0.8	2.0

not be examined, because these resulted in a complete loss of cells after 24 h. In contrast to the previously mentioned σ-ligands, AC915 in doses of up to 776 μM did not increase cellular ^{18}F-FDG uptake. A slight but statistically significant decline (−21%) of ^{18}F-FDG uptake was observed after incubation of cells with 220 μM AC915 (Fig. 3).

Treatment of C6 cells with rimcazole, (+)-pentazocine, and haloperidol resulted in a dose-dependent depression of ^{18}F-FLT uptake when drug concentrations exceeded threshold values of 20, 50, and 80 μM, respectively (Fig. 4). Strong and statistically significant depression of ^{18}F-FLT uptake was observed after a 24-h incubation of the cells with 50 (rimcazole), 100 (haloperidol), or 645 μM ((+)-pentazocine), to 6%, 17%, and 26% of the control, respectively. In contrast to the previously mentioned σ-ligands, AC915 did not depress cellular ^{18}F-FLT uptake but caused a biphasic increase with a maximum occurring at a drug dose of 74 μM (Fig. 4).

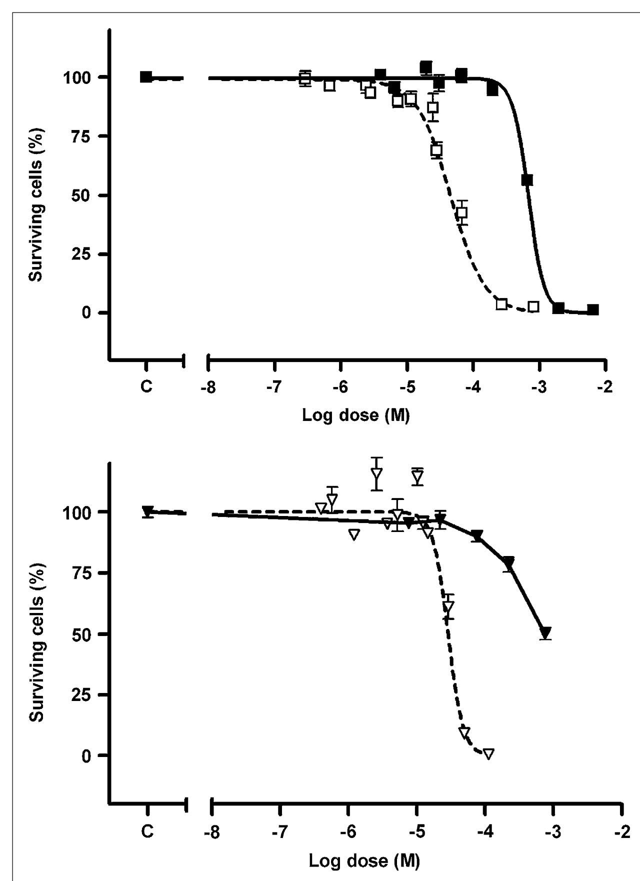

FIGURE 2. Dose-dependent cell loss after 24 h of incubation with (+)-pentazocine (■), haloperidol (□), AC915 (▼), and rimcazole (▽). Note that *x*-axis scales of Figures 1 and 2 are different. Error bars indicate SEM.

Cellular uptake of ^{11}C-choline was depressed by all drug treatments (Fig. 5). A decline occurred when the ligand concentration of (+)-pentazocine and AC915 exceeded 70 μM (20 μM for haloperidol and 15 μM for rimcazole). At concentrations of 650 ((+)-pentazocine), 711 (AC915), or 45 μM (rimcazole), ^{11}C-choline uptake was reduced more than 80%, whereas a concentration of 60 μM for haloperidol resulted in a 55% decline (Fig. 5).

No statistically significant changes of ^{11}C-methionine uptake occurred after 24 h of treatment of C6 cells with (+)-pentazocine, haloperidol, or AC915 (Fig. 6). In contrast, rimcazole at concentrations greater than 10 μM depressed uptake of the amino acid up to 40% (Fig. 6).

DISCUSSION

Competition Assays

^{11}C-SA4503 has been reported to bind preferentially to σ-1 receptors (IC_{50}, 17.4 nM at the σ-1 and 1,784 nM at the σ-2 subtype (*27*); IC_{50}, 4.7 nM at σ-1 and 63.1 nM at σ-2 receptors in a later study (*28*)). Therefore, we expected that the potent σ-1 selective ligands (+)-pentazocine and AC915 would compete more efficiently (i.e., at lower IC_{50} values) with cellular binding of ^{11}C-SA4503 than would the non–subtype-selective antagonists rimcazole and haloperidol, which bind less potently to σ-1 receptors. In reality, however, rimcazole and haloperidol inhibited ^{11}C-SA4503 binding more potently (IC_{50} values, 0.36 and 0.27 μM, respectively) than did (+)-pentazocine and AC915 (IC_{50} values, 6.5 and 7.4 μM, respectively; Fig. 1). The IC_{50} values corresponded closely to the σ-2 K_d but not to the σ-1 K_d of the test compounds (Table 1).

This outcome of our competition assays can be understood when the applied mass of ^{11}C-SA4503 is considered.

TABLE 2
Receptor Occupancy at Cytotoxic Dose

Test drug	IC_{50} for inhibition of ^{11}C-SA4503 binding in C6 cells (μM)	EC_{50} for cell killing (within 24 h; μM)	EC_{50}/IC_{50}	Receptor occupancy (%)
AC915	7.4	819	110	99.1
(+)-Pentazocine	6.5	710	109	99.1
Haloperidol	0.27	58	215	99.5
Rimcazole	0.36	31	86	98.9

To have acceptable count statistics even at high doses of a competing drug after 60 min of incubation, it was necessary to add 4 MBq of ^{11}C-SA4503. At a specific radioactivity of 22 TBq/mmol, this corresponds to a ligand mass of 0.2 nmol and a ligand concentration of 200 nM in each well. The receptor K_d reported by Matsuno et al. (*27*) predict that at this radioligand concentration, saturation of σ-1 receptors will occur and specific binding will be observed mainly at the σ-2 subtype.

Thus, the methods described in this article investigated the relationship between cytotoxicity and σ-2 receptor occupancy, using the relatively low affinity of ^{11}C-SA4503 for σ-2 receptors. During in vivo scans with ^{11}C-SA4503 in humans, ligand concentrations in the 10^{-8} or 10^{-7} M range will never be reached. Human SA4503-PET scans will, therefore, reflect the regional distribution of σ-1 rather than σ-receptors.

Cytotoxic Effects

According to the literature, relatively high doses of σ-antagonists (20–100 μM for rimcazole or haloperidol (*12,13*)) are required for the killing of glioma cells. Our

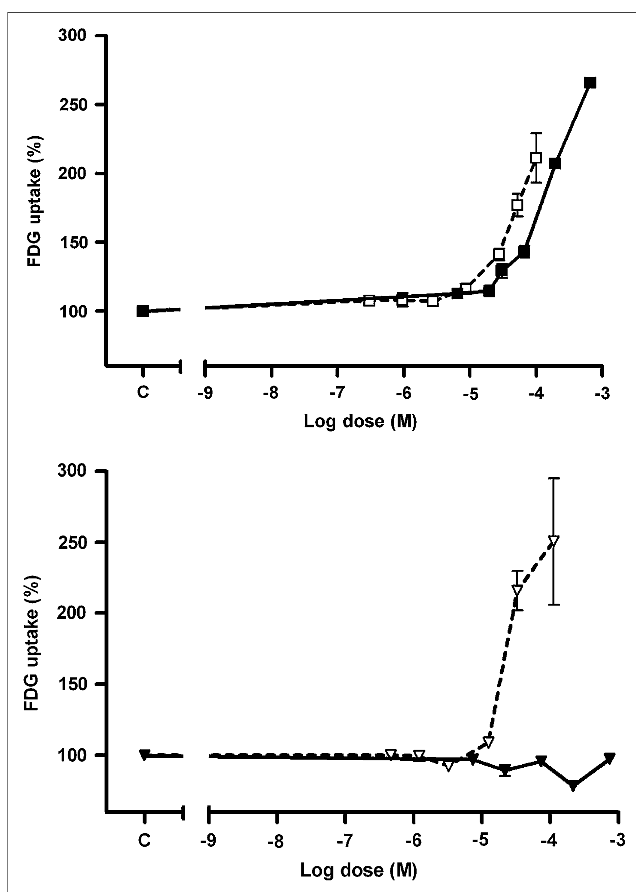

FIGURE 3. Dose-dependent increase of cellular ^{18}F-FDG uptake by (+)-pentazocine (■), haloperidol (□), AC915 (▼), and rimcazole (▽).

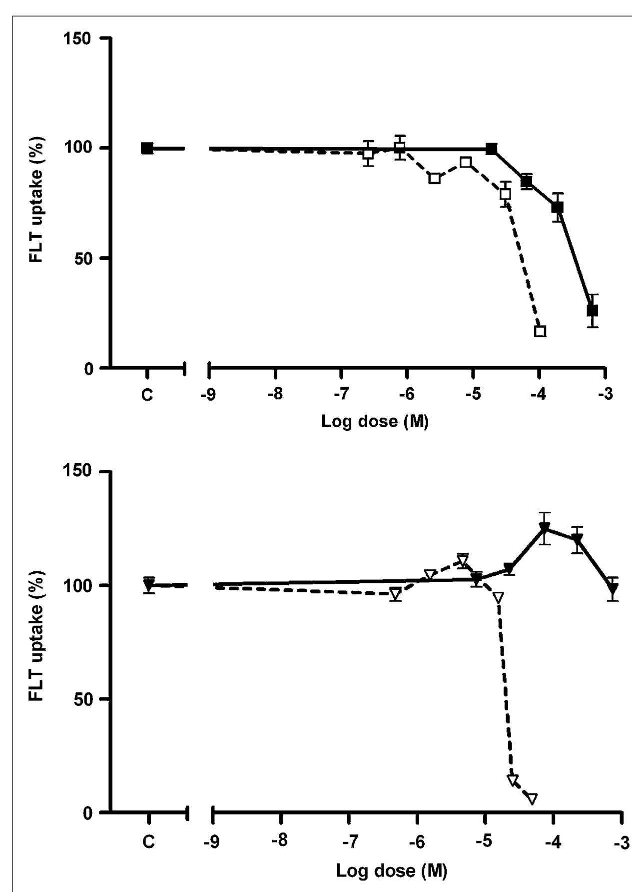

FIGURE 4. Dose-dependent inhibition of cellular ^{18}F-FLT uptake by (+)-pentazocine (■), haloperidol (□), AC915 (▼), and rimcazole (▽).

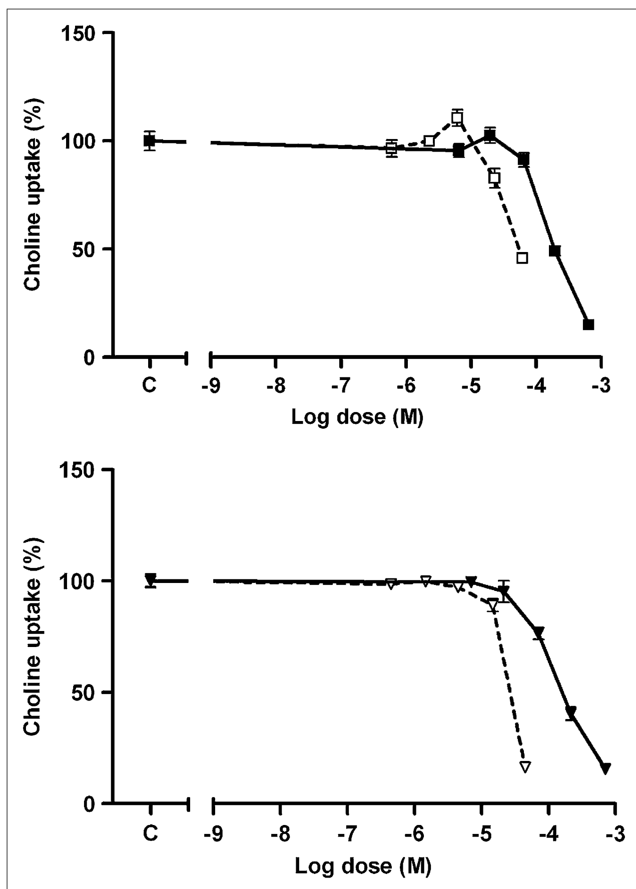

FIGURE 5. Dose-dependent inhibition of cellular ^{11}C-choline uptake by (+)-pentazocine (■), haloperidol (□), AC915 (▼), and rimcazole (▽).

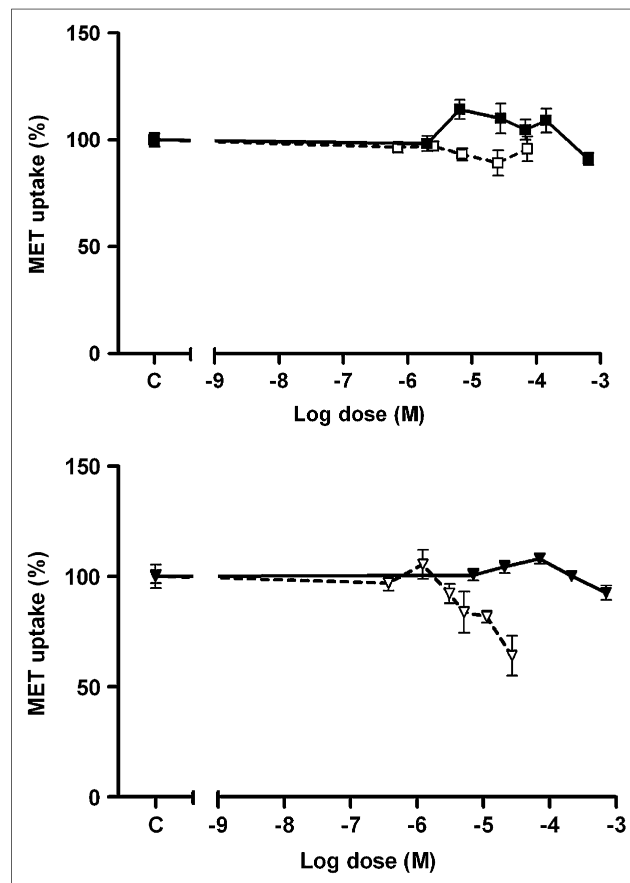

FIGURE 6. Dose-dependent inhibition of cellular ^{11}C-methionine uptake by (+)-pentazocine (■), haloperidol (□), AC915 (▼), and rimcazole (▽). MET = methionine.

data confirm these reports (EC_{50}, 31 μM for rimcazole and 58 μM for haloperidol; Fig. 2). Even higher doses of (+)-pentazocine and AC915 were necessary to induce cell death (EC_{50} values, 710 and 819 μM, respectively; Fig. 2). Such high doses are not required because diffusion barriers limit drug access to intracellular sites, for half-maximal occupancy of the σ-receptor population is reached already at concentrations 100-fold lower than the EC_{50} for cell killing (Table 2). Because we observed a decrease in cellular viability only at drug concentrations 2 orders of magnitude higher than the corresponding IC_{50} values for inhibition of ^{11}C-SA4503 binding, our data indicate that cell death is associated with virtually complete (>98%) occupancy of the σ-receptor population. Because ^{11}C-SA4503 binding occurred mainly at σ-2 receptors under the conditions of our assay, the data also suggest that cell death is induced via the σ-2 rather than via the σ-1 subtype if the observed cytotoxic effects are really mediated through σ-receptors.

The curves describing cell loss after 24 h of treatment with rimcazole and (+)-pentazocine have a steep slope, indicating rapid cell killing when the drug dose exceeds a sharp threshold concentration (Fig. 2). In contrast, cell death induced by haloperidol and AC915 occurs more gradually (Fig. 2). These different curve shapes may signify that haloperidol and AC915 are partial agonists at σ-2 receptors, whereas rimcazole and (+)-pentazocine are full agonists. It is also possible that rimcazole and (+)-pentazocine induce cell death via an additional mechanism, which occurs in parallel and independently of occupancy of the σ-2 receptor population. σ-2 receptor–mediated cell death is suggested by the fact that losses of cell viability always occur at concentrations 2 orders of magnitude higher than the IC_{50} of the 4 test compounds for inhibition of ^{11}C-SA4503 binding to cellular σ-2 receptors (Table 2). An additional mechanism of cell death is suggested by the different shapes of the cell-loss curves (Fig. 2) and by a cellular morphology that is different after treatment of the cells with different σ-ligands (data not shown).

On the basis of these data, rimcazole appears to be the most interesting compound for further study as an anticancer drug.

Metabolic Changes

In our in vitro model, we observed a large increase of ^{18}F-FDG uptake after 24 h of incubation with σ-receptor ligands (Fig. 3). A similar increase of ^{18}F-FDG uptake has

been observed after some forms of chemo- and radiotherapy in which the increase of uptake preceded cell death (*29,30*). This increase may be a "metabolic flare" due to increased energy consumption as a consequence of DNA and membrane repair (*29*). Glucose transporters (glucose transporter 1 and sodium-dependent glucose transporter 1) are known to be overexpressed after various cellular stresses, including hypoxia and heat shock (*31*). Rimcazole and (+)-pentazocine interfered strongly with cellular ^{18}F-FDG uptake, whereas haloperidol and AC915 had smaller effects on glucose metabolism (Fig. 3).

^{18}F-FLT traces the salvage pathway of DNA synthesis. The retention of this nucleoside is a measure of thymidine kinase 1 activity within the cells (*32,33*). After treatment of C6 cells with σ-receptor ligands, we observed striking decreases of ^{18}F-FLT uptake. (+)-Pentazocine, rimcazole, and haloperidol induced more than a 70% decline of cellular ^{18}F-FLT uptake at a dose 100 times greater than the IC_{50} for inhibition of cellular ^{11}C-SA4503 binding (Fig. 4). However, AC915 caused a less marked inhibition of ^{18}F-FLT uptake, which is consistent with a smaller reduction of cellular proliferation (Fig. 4). The rank order of drug effects on ^{18}F-FLT uptake was the same as for ^{18}F-FDG. In the range of 3–30 times IC_{50} for inhibition of cellular ^{11}C-SA4503 binding, AC915 induced a small increase of ^{18}F-FLT uptake, which may reflect activation of repair processes within the cells.

We tested ^{11}C-choline uptake after treatment of C6 cells with σ-receptor ligands because total choline levels and phosphatidylcholine synthesis are known to be elevated in cancers of different origin, as compared with healthy tissue, and are related to cellular proliferation. We observed the expected strong decrease of ^{11}C-choline uptake when cells were treated with σ-ligands (Fig. 5). Above the threshold rimcazole concentration, the uptake of ^{11}C-choline was reduced rapidly, whereas (+)-pentazocine, AC915, and haloperidol resulted in slower but progressive inhibition of phospholipid metabolism. These results are consistent with the fact that ^{11}C-choline uptake by cancer cells is related to the activity of the enzyme choline kinase (*34*), which is generally directly proportional to the proliferative status of the cell.

In contrast to ^{18}F-FLT and ^{11}C-choline, ^{11}C-methionine did not behave as a proliferation marker, and ^{11}C-methionine uptake per viable cell showed hardly any change (Fig. 6). Thus, ^{11}C-methionine appeared to trace cell number rather than cellular viability after σ-ligand treatment. The methyl group of methionine, which contains the ^{11}C label, is implicated in many metabolic processes such as protein synthesis, transmethylation, and polyamine synthesis. Therefore, ^{11}C-methionine has a complex metabolism, and uptake of ^{11}C-methionine by tumor cells may be a reflection of several processes, which are affected differently by anticancer treatment, some being increased and others showing a decrease (*35*). The lack of change in ^{11}C-methionine uptake may also indicate unaltered transport of this amino acid through the cell membrane and slow transmethylation processes (*31*).

Our data indicate that ^{18}F-FLT and ^{11}C-choline are the tracers of choice for evaluation of the effects of σ-ligands on cellular proliferation. ^{18}F-FDG may also provide useful information, but ^{11}C-methionine uptake is a reflection of the cell number rather than the proliferation state.

Growth reduction of glioma cells and changes of cellular metabolism were observed only at ligand concentrations more than 30-fold higher than those required for 50% occupancy of the σ-2 receptor population. Strong decreases of the number of viable cells occurred at drug concentrations greater than or equal to 100-fold higher than the IC_{50} for inhibition of ^{11}C-SA4503 binding. Thus, cell death was associated with virtually complete (99%) occupancy of the intracellular σ-2 receptor pool.

Although high levels of receptor occupancy and drug doses in the 10^{-5} M range are required for rapid cell killing, σ-ligands appear to have potential as cancer therapeutics for the following reasons. First, preliminary studies, both from our own laboratory and from other institutions (*18*), have demonstrated that cell death is limited to tumor cells and occurs only at much higher concentrations in healthy normal cells. Adverse effects could be further diminished by local application or selective drug targeting. Second, significant inhibition of in vivo tumor growth has been observed in a variety of tumor models in response to σ-ligands given systemically (*17,18*). Third, because σ-2 ligands trigger cell death both by caspase-dependent and caspase-independent mechanisms, such compounds may be suitable for treatment of a wide variety of tumors, including caspase-deficient tumor types (*36*). Last, subtoxic doses of σ-ligands can be applied in combination therapy and can potentiate the antitumor effects of "classic" cytostatic agents (*36,37*). Because of the therapeutic potential of σ-ligands, radiopharmaceuticals for σ-receptors may be used not only as diagnostic imaging agents but also as tools to assess σ-receptor occupancy during experimental tumor therapy.

CONCLUSION

In the present study, treatment of tumor cells with cytotoxic doses of σ-ligands resulted in strong increases of the uptake of ^{18}F-FDG and decreases of ^{18}F-FLT and ^{11}C-choline uptake per viable cell. Cell-specific uptake of ^{11}C-methionine was largely unaltered under these conditions. ^{18}F-FLT, ^{11}C-choline, and ^{18}F-FDG responded strongly to drug treatment and were suitable tools in the evaluation of the cytotoxicity of the test compounds. These PET tracers may, therefore, be used in future in vivo studies.

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