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European Journal of Pharmacology 278 (1995) 151–160

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σ Binding site ligands inhibit cell proliferation in mammary and colon carcinoma cell lines and melanoma cells in culture

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Received 27 October 1994; revised 9 February 1995; accepted 17 February 1995

Abstract

Recent evidence suggests a role for sigma (σ) binding sites in maintenance of cell growth and/or proliferation. The present study examines, for the first time, the effect of σ binding site ligands on in vitro growth of tumour cells derived from human mammary adenocarcinoma (MCF-7, MDA) and colon carcinoma (LIM 1215, WIDr), and melanoma (Chinnery). Addition of the σ ligands haloperidol, reduced haloperidol, 1,3-di(2-tolyl)guanidine (DTG), (+)- and (−)-*N*-allylnormetazocine (SKF 10,047), (+)- and (−)-pentazocine and rimcazole at 6.25, 12.5, 25, 50, 100 μM at the beginning of culture or 24 h later, inhibited cell proliferation in a dose-dependent manner. Light microscopy revealed cell detachment, rounding and cell death. The potency of σ ligands on melanoma cells was rimcazole > reduced haloperidol > haloperidol = (+)-pentazocine, whereas DTG and (+)- and (−)-SKF 10,047 and (−)-pentazocine had no effect even at 100 μM. In contrast, in MCF-7 cells, rimcazole > reduced haloperidol > haloperidol > (−)-pentazocine > DTG > (+)-pentazocine > (+)-SKF 10,047 > (−)-SKF 10,047. For colon cancer cells, reduced haloperidol > DTG > haloperidol = (−)-pentazocine = (+)-pentazocine = (+)-SKF 10,047. Of all the ligands tested, rimcazole and reduced haloperidol were the most potent inhibitors of cell proliferation. With the exception of one slow-growing colon cancer cell line (LIM 1215), the order of sensitivity of various cell lines to reduced haloperidol, SKF 10,047, DTG, haloperidol and (+)- and (−)-pentazocine was colon carcinoma > mammary adenocarcinoma > melanoma, whereas to rimcazole, the sensitivities of mammary adenocarcinoma and melanoma cells were comparable. The effect of σ ligands in MCF-7 and melanoma cells was not due to blockade of dopamine D_1 and D_2 receptors, serotonin ($5\text{-}HT_2$) receptors, *N*-methyl-D-aspartate (NMDA)/phencyclidine receptors, β-adrenoceptors or opioid receptors, since 100 μM SCH 23390, raclopride, mianserin, (+)-MK-801, propranolol and 1 μM naloxone respectively, were ineffective. However, mianserin and raclopride were inhibitory to melanoma cells and one colon carcinoma cell line, respectively. Taken together, the results are consistent with the recent observation that σ binding sites may play a role in cell growth and/or cell proliferation.

Keywords: Receptor ligand; Cell proliferation; Mammary carcinoma cell; Colon carcinoma cell; Melanoma cell

1. Introduction

Sigma (σ) receptors were initially proposed by Martin and colleagues (1976) to represent a new category of opiate receptors (for reviews see Quirion et al., 1992; Walker et al., 1990). Subsequent work, however, has led to a firm distinction between σ and opioid recognition sites. These receptors have a unique pharmacological profile and brain distribution when compared with other receptors. Two subtypes of σ receptors, σ_1 and σ_2, exist, and evidence suggests that σ receptors are functionally important (Quirion et al., 1992; Walker et al., 1993; Bowen, 1993). Sigma receptors are widely distributed in diverse peripheral tissues such as liver, kidney, gastrointestinal tract and endocrine glands such as ovary, adrenal, testis and pituitary (Su et al., 1988; Roman et al., 1988; Samovilova et al., 1988; Riviere et al., 1990; Bowen, 1993), as well as in leucocytes (Wolfe et al., 1988), and interact with cell signalling pathways which contain proto-oncogene products and regulate cell proliferation (Vilner and Bowen, 1993; Bowen, 1993; Brent et al., 1994; Bunn et al., 1994; Vilner et al., 1994).

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Elsevier Science B.V.
SSDI 0014-2999(95)00115-8

Several rodent and human clonal cell lines of neuronal and non-neuronal origin have been reported to contain high densities of σ sites, including NCB-20 cells, neuroblastomas NB41A3, N1E-115, S20Y, glioma C6, neuroblastoma-glioma hybrid cell line NG108-15, SK-N-SH and SH-SY5Y neuroblastomas, COS-7 (kidney), MRS-5 (lung) and PC12 pheochromocytoma (Yang et al., 1989; Vilner and Bowen, 1992, 1993; Bowen, 1993; Vilner et al., 1994, 1995). The presence of σ binding sites in cultured cell lines provides model systems consisting of a homogeneous cell type in which to study the function of σ sites. This provides an advantage over central and peripheral tissues which consist of multiple cell types containing a heterogeneous population of σ sites (see Bowen, 1993).

Recent studies demonstrated that σ-active drugs had profound effects on cell morphology and viability in C6 glioma cells, causing loss of processes, assumption of spherical shape, and cessation of cell division, whereas σ-inactive compounds did not (Vilner and Bowen, 1993; Vilner et al., 1994). Furthermore, nude mice-borne C6 glioma cells displayed a doubling of their total σ binding after extended passaging (Barg et al., 1994), consistent with previously observed overexpression of σ receptors in human transformed renal and colorectal carcinomas and meningiomas, when compared to normal cells of the same origin (Thomas et al., 1990; Bem et al., 1991; Bowen, 1993). Moreover, recent findings that σ receptors are expressed in human malignant melanoma (John et al., 1993a,b; 1994; Vilner et al., 1995), non-small cell lung carcinoma (John et al., 1995b), leukemia, prostate and glioblastoma (Vilner et al., 1995), and in MCF-7 mammary adenocarcinoma (John et al., 1995a; Vilner et al., 1995), have led to the development and characterization of new σ receptor radiopharmaceuticals that could be used as external markers for imaging and molecular characterization of tumours. For example, it has been demonstrated that labelled σ receptors could be used as external markers for imaging malignant melanoma tumoral xenografts in nude mice models (John et al., 1993b), and the novel iodinated σ receptor ligand, *N*-(2-diethylaminoethyl-4-iodobenzamide ([^{123}I]DAB), has been successfully used to image malignant melanoma in clinical trials in humans (Michelot et al., 1993).

These results demonstrating the expression of σ binding sites by several clonal cell lines and human tissues, strongly suggest that σ receptors play a role in cell growth and development. In view of the expression of σ binding sites by several clonal cell lines and human tissues, and in order to examine the possible involvement of σ binding sites, we have investigated the effect of various σ and non-σ compounds, on human mammary and colon carcinoma cell lines and melanoma cells in culture. These cell lines originate from different tissue sources (mucosal, skin) and have different growth rates. A role for σ binding sites in the regulation of cell proliferation in these carcinoma cell lines has not been studied. In this study, we demonstrate that σ compounds can inhibit the in vitro growth of tumour cells derived from human mammary and colon carcinoma, and melanoma. Our study also shows a pattern of differential activity of σ ligands against all tumour cells. In general, tumour cell lines with high growth rate and of different tissue origin respond differently to σ ligands, suggesting that σ binding sites may play an important role in modulating the growth of tumour cells at different tissue sites in vitro.

2. Materials and methods

2.1. Cell culture

Human mammary (MCF-7) adenocarcinoma cell lines, MCF-7, which was originally isolated from a pleural effusion of a primary breast cancer patient (Soule et al., 1973), and MDA, and colon carcinoma cell line WIDr, were obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). LIM 1215 established from colon carcinoma was kindly provided by Dr. R. Whitehead (Ludwig Institute of Cancer Research, Melbourne, Australia). Melanoma cell line (Chinnery) was established from skin lesions of a patient with melanoma (Chen and Hersey, 1991). The cells were cultured as a monolayer in a 25 cm^2 tissue culture flask containing Dulbecco's modified Eagle's medium (DMEM, Commonwealth Serum Laboratories, Melbourne, Australia) supplemented with 10% foetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin in an atmosphere of 5% CO_2 in air. When cells reached 60–70% confluency they were removed by treatment with trypsin-versene solution and the cells obtained were washed in DMEM before they were used in the growth inhibition assay described below.

2.2. Cell growth inhibition assay

The effect of σ ligands on cell proliferation in the cancer cell lines was determined using the tetrazolium-based compound MTT (3-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan) (Carmichael et al., 1987). MCF-7, LIM 1215, WIDr or melanoma cells were suspended at 2.5×10^4 cells per ml in complete DMEM. The cells were then seeded in 100 μl aliquots into wells of a 96-well flat-bottomed microtitre plate. Stock solutions of ligands used (10 mM) were dissolved in DMEM to the required working concentrations. To each well, 100 μl DMEM containing the σ binding site ligands haloperidol, reduced haloperidol, 1,3-di(2-

tolyl)guanidine (DTG), (+)- and (−)-*N*-allylnormetazocine (SKF 10,047), (+)- and (−)-pentazocine and rimcazole at various concentrations (6.25–100 μM), or propranolol, SCH 23390, raclopride, MK-801, mianserin (100 μM respectively) and naloxone (1 μM), was added in triplicates either at the beginning of culture or 24 h later. The concentration of naloxone used (1 μM) was 200 times higher than that used to block the inhibitory effect of opioids on cell proliferation in MCF-7 cells in a previous study (Maneckjee et al., 1990). Control cultures contained cells with medium alone. The cultures were incubated for 48 h, after which time 10 μl of MTT (500 μg/ml) dye was added to each well. After incubation for a further 4 h, 100 μl of the medium was removed from each well and replaced with 100 μl of isopropanol to extract the insoluble product resulting from conversion of the MTT dye by viable cells. Thus, the number of proliferating cells in each well is proportional to the intensity of the blue colour reaction which was then read in an ELISA plate reader using a 595 nm filter. The results were expressed as percentages of control cultures without ligand and each value obtained was calculated using the mean triplicate cultures for each experimental group. Cultures were also examined visually under an inverted microscope and photographed (Zeiss, Germany).

2.3. Drugs and solutions

The following drugs were used: 1,3-(2-tolyl)guanidine (DTG) and naloxone hydrochloride (Sigma Chemical Company, USA); (+)- and (−)-SKF 10,047 (*N*-allylnormetazocine), reduced haloperidol, mianserin hydrochloride, SCH 23390 hydrochloride, (+)- and (−)-pentazocine (Research Biochemicals, USA), raclopride (Astra Pharmaceuticals, Sweden), propranolol hydrochloride (ICI Pharmaceuticals, Australia) and haloperidol (Searle Laboratories, Australia).

3. Results

3.1. Light microscopy

Microscopic examination after treatment of mammary and colon carcinoma cells and melanoma cells with σ ligands revealed rounding of cells, cell detachment, and ultimately cell death. The degree of these effects on cell proliferation was dependent on the type and dose of σ ligand used. Without treatment, human mammary and colon carcinoma cells and melanoma cells were fusiform or spindle in shape and were tightly attached to the plastic surface. Colon carcinoma cells grew in colonies (see Fig. 1F for representative WIDr cells). After treatment with σ ligands, many rounded dying cells were seen either as single cells or in clumps which no longer adhered to the plastic surface (see MCF-7 cells treated with rimcazole (50 and 100 μM; Fig. 1B and C) and reduced haloperidol (50 and 100 μM; Fig. 1D and E), and WIDr cells treated with rimcazole (50 and 100 μM; Fig. 1G and H) and reduced haloperidol (50 and 100 μM; Fig 1I and J)). Similar effects were obtained for σ ligands in other cell lines (not shown).

3.2. Cell proliferation assay

Melanoma cells

Cell proliferation was inhibited 2 days after addition of σ ligands to melanoma cells at time of seeding. The order of potency at the maximum dose tested (100 μM) was: reduced haloperidol (86%) > haloperidol (20%) > (+)-pentazocine (17%). DTG, (+)- and (−)-SKF 10,047 and (−)-pentazocine had no effect at 100 μM (Fig. 2). No effects on cell proliferation were found with 100 μM of SCH 23390, raclopride, MK-801, propranolol, or 1 μM naloxone, in melanoma cells, although mianserin produced 30% inhibition only at 100 μM in this cell line with no effect at 6.25–50 μM, suggesting that this effect of mianserin may have been non-specific. In one set of experiments using 5000 cells/well instead of 2500 cells/well, similar results were obtained for σ ligands (100 μM): reduced haloperidol (73%), haloperidol (65%), (+)-pentazocine (22%) and (−)-pentazocine (0%). In a separate set of experiments, the effects of the selective σ ligand, rimcazole, on cell proliferation in melanoma cells was examined. Rimcazole potently inhibited cell proliferation in melanoma cells (37–98% at 25–100 μM respectively, Table 1). In experiments where σ ligands were added 24 h after seeding, similar results to those above were obtained (not shown).

Human mammary carcinoma (MCF-7) cells

Cell proliferation was inhibited dose-responsively 2 days after addition of σ ligands to MCF-7 mammary carcinoma cells at time of seeding. The order of potency at the maximum dose tested (100 μM) was: reduced haloperidol (98%) > haloperidol (83%) > (−)-pentazocine (58%) > DTG(40%) > (+)-pentazocine (29%) > (+)-SKF 10,047 (22%) = (−)-SKF 10,047 (23%) (Fig. 3). These effects in MCF-7 cells were not due to blockade of dopamine D_1 and D_2 receptors, 5-HT_2 receptors, NMDA/PCP receptors, β-adrenoceptors or opioid receptors, since 100 μM of SCH 23390, raclopride, mianserin, MK-801, propranolol and 1 μM naloxone, respectively, were inactive (data not shown). In addition, there was no difference to inhibition of cell growth produced by (+)- and (−)-pentazocine (100 μM) in the presence of naloxone (1 μM). In these experiments, naloxone (1 μM) in combination with (+)-pentazocine (100 μM) produced a small (10%)

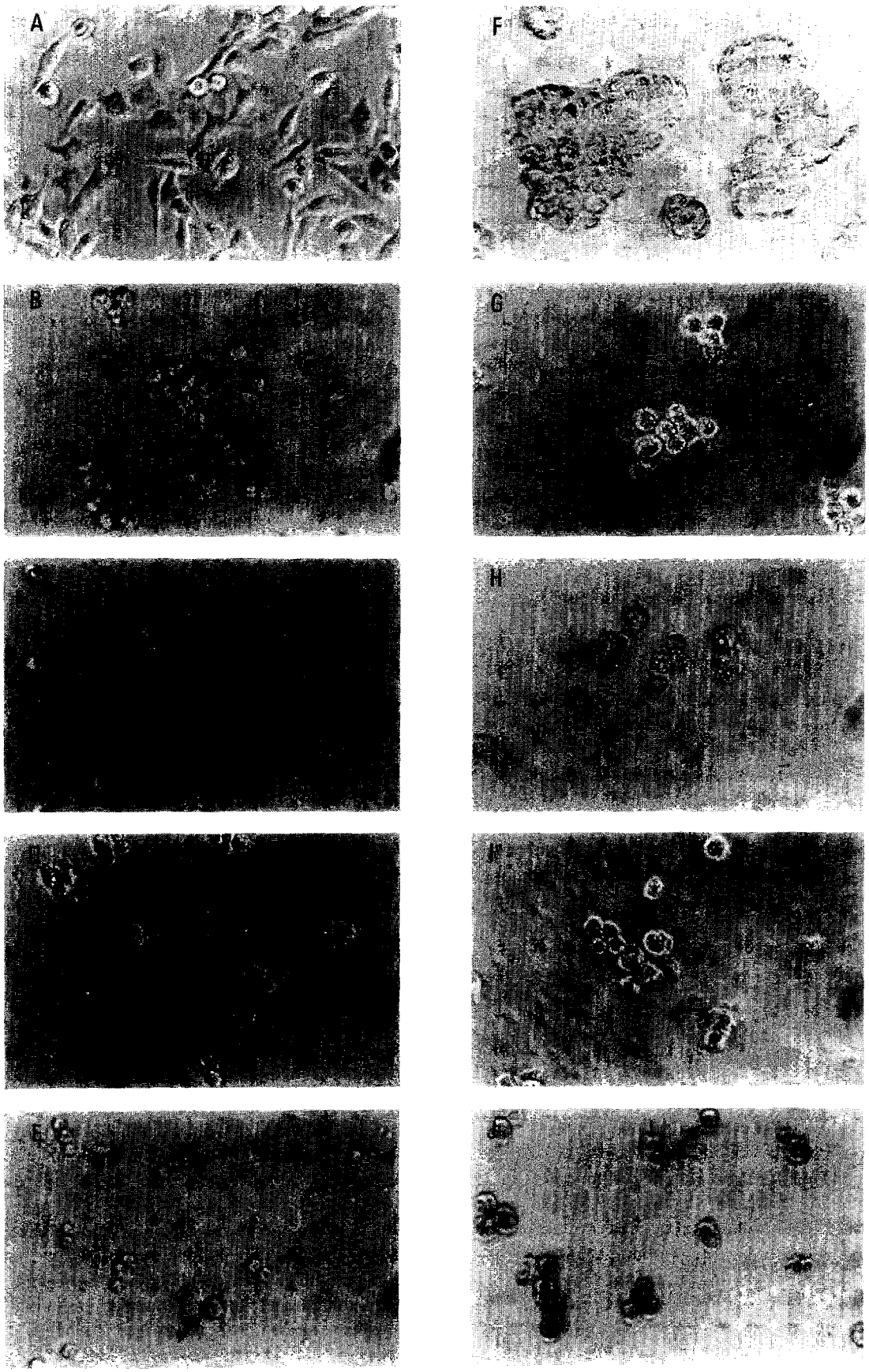
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Table 1
Effects of rimcazole on cell proliferation in MCF-7 carcinoma and melanoma cells

Cell line	μM	% Inhibition
MCF-7	100	97±1.2
	50	89±2.4
	25	37±3.3
	12.5	0±0
	6.25	0±0
Melanoma	100	98±0.9
	50	93±1.1
	25	24±4.3
	12.5	0±0
	6.25	0±0

Cells were cultured at 2500 cells/well in the presence or absence of σ ligands. After 48 h the cultures were assessed for growth by the MTT assay. Data shown are expressed as percentage inhibition of cell proliferation (mean±S.E.M., triplicate determinations; $n=3$ experiments) and were normalised to 100% of control cultures without drug.

increase in inhibition of cell proliferation compared with (+)-pentazocine alone (40% inhibition for (+)-pentazocine in combination with naloxone, compared to 30% for (+)-pentazocine alone), whereas naloxone in combination with (−)-pentazocine made no difference (0%) compared to the inhibitory effect of (−)-pentazocine alone, thus ruling out opioid effects of (+)-pentazocine and (−)-pentazocine. In another mammary carcinoma cell line examined, MDA, the order of potency at 100 μM was: reduced haloperidol (72%) > (−)-pentazocine (55%) > DTG (37%) = haloperidol (34%) > (+)-pentazocine (25%) > (−)-SKF 10,047 (21%) > (+)-SKF 10,047 (12%) (Table 2). In separate experiments, rimcazole also produced potent inhibition of cell proliferation in MCF-7 carcinoma cells (24–97% at 25–100 μM respectively; Table 2). Similar effects were obtained when σ ligands were added 24 h after seeding (not shown).

Colon carcinoma cells

Cell proliferation was inhibited dose-responsively 2 days after addition of σ ligands to colon carcinoma cells (LIM 1215 and WIDr) at time of seeding. The order of potency at the maximum dose tested (100 μM) for LIM 1215 was: reduced haloperidol (63%) > DTG (47%) > haloperidol (32%) > (+)-pentazocine (22%) = (+)-SKF 10,047 (20%) > (−)-pentazocine (17%) (Fig. 4). For WIDr cells, the order of potency at the maximum dose tested was: reduced haloperidol (92%) = haloperidol (90%) > (+)-pentazocine (70%) > DTG (51%) = (−)-pentazocine (44%) > (+)-SKF 10,047 (33%) = (−)-SKF 10,047 (30%). Similar results were obtained when drugs were added 24 h after seeding (not shown). There was no effect of 100 μM SCH 23390, raclopride, mianserin, MK-801 or propranolol, or 1 μM naloxone in LIM 1215 cells, but in WIDr cells, the dopamine D_2 receptor antagonist, raclopride, produced significant inhibition of cell proliferation (30–70% inhibition for 6.25–100 μM respectively).

Comparison of growth inhibitory effects of σ ligands on tumour cells established from different tissues

Table 2 shows the relative sensitivity to σ ligands of various cell lines established from different anatomical sites. In terms of the proliferative response, colon (WIDr, doubling time 15 h) and mammary (MCF-7, doubling time 16 h) carcinoma cell lines were more sensitive to σ ligands than melanoma cells (Chinnery, doubling time 17–24 h). The slow growing colon cancer line (LIM 1215, doubling time 32 h), and the slow growing mammary cancer line (MDA, doubling time 28 h) were comparable, but with exception to reduced haloperidol, they were more sensitive in a dose-dependent fashion to haloperidol, DTG, (+)- and (−)-SKF 10,047 and (+)- and (−)-pentazocine compared with melanoma cells.

4. Discussion

In the present study, we have used the well characterized human mammary carcinoma cell line, MCF-7, together with another mammary line, MDA, and two colon cell lines, LIM 1215 and WIDr, derived from separate patients with colon cancer, as well as a melanoma cell line established from a skin lesion of a patient with melanoma, to determine the ability of various selective σ ligands to modulate growth of these cells in culture. Sigma binding site ligands, when added to human mammary and colon carcinoma and melanoma cells in culture, caused marked inhibition of

Fig. 1. Phase contrast microscopy of mammary adenocarcinoma (MCF-7) and colon carcinoma (WIDr) cells cultured with or without the σ ligands rimcazole (50 and 100 μM) and reduced haloperidol (50 and 100 μM) as described in Materials and methods. A and F: Control MCF-7 and WIDr culture as they appear before addition of any test compound, containing tightly adherent cells of cuboidal or spindle shape (MCF-7), or growing in colonies (WIDr). B–E: Culture (MCF-7) treated with rimcazole (50 μM (B) and 100 μM (C)) and reduced haloperidol (50 μM (D) and 100 μM (E)) and photographed 48 h after addition of drugs. G–J: Culture (WIDr) treated with rimcazole (50 μM (G) and 100 μM (H)) and reduced haloperidol (50 μM (I) and 100 μM (J)) and photographed 48 h after addition of drugs. Morphological changes in MCF-7 and WIDr cells produced by σ ligands consisted of cell-cell detachment, cell rounding (single or in groups – filled arrows) and cell death (magnification × 100). Photographs are from representative experiments.

cell proliferation, whereas σ-inactive compounds which act at dopamine D_1 and D_2, 5-HT_2 and NMDA/PCP receptors, or opioid receptors and β-adrenoceptors, had little or no effect. Furthermore, the active ligands used in the present study are from different structural classes. Taken together, these data would argue against a non-specific effect, and add more support to the notion of a common cellular mechanism. The results for the effects of σ ligands on cell proliferation in human mammary and colon carcinoma cells and melanoma cells in the present study are consistent with recent data demonstrating the presence of σ binding sites in similar cell lines (Bem et al., 1991; John et al., 1993a,b, 1994, 1995a; Barg et al., 1994; Vilner et al., 1995).

The relative potency of the various σ ligands tested on cell proliferation was generally consistent with their affinity and/or selectivity at σ binding sites, suggesting mediation of these effects by σ sites. For example, in the present studies, the selective σ ligand rimcazole potently inhibited cell proliferation in the two cell lines examined with this drug. The explanation for this result may lie in the high selectivity of rimcazole for σ sites (Ferris et al., 1982). Furthermore, in MCF-7 cells for example, with the exception of rimcazole, which has moderate affinity for σ sites (Ferris et al., 1986), the order of potency for the various ligands tested was reduced haloperidol > haloperidol > DTG, consistent

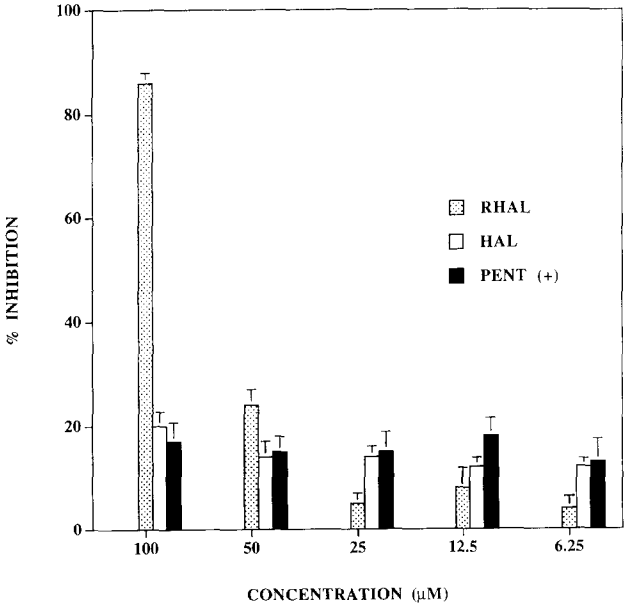

Fig. 2. Growth-inhibitory effect of σ ligands reduced haolperidol, haloperidol and (+)-pentazocine in melanoma cells. Cells were cultured at 2500 cells/well in the presence of σ ligands. After 48 h the cultures were assessed for growth by the MTT assay. Results shown (mean ± S.E.M. of triplicate determinations; $n = 3$) are normalised to 100% of control cultures without drug. The σ ligands (+)- and (−)-SKF 10,047, DTG and (−)-pentazocine had no effect on cell proliferation in melanoma cells.

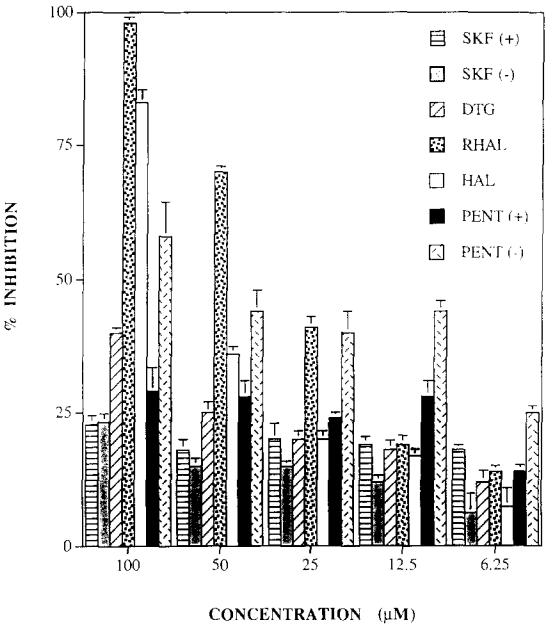

Fig. 3. Growth-inhibitory effect of σ ligands in MCF-7 mammary carcinoma cells. Conditions as for Fig. 2. Results shown (mean ± S.E.M., triplicate determinations; $n = 3$) are normalised to 100% of control cultures without drug.

with the binding affinities of reduced haloperidol, haloperidol and DTG (σ K_i = 5.1 nM, 3.7 nM and 28 nM respectively) obtained from studies in guinea-pig brain (see Vilner and Bowen, 1993). Dose-related effects could be demonstrated in MCF-7 cells for rimcazole, reduced haloperidol, haloperidol, DTG and (−)-pentazocine (and similarly for reduced haloperidol, haloperidol, DTG and (−)-pentazocine in MDA cells), whereas in colon carcinoma cells (LIM 1215), the effects of reduced haloperidol, haloperidol and DTG were dose-related, and in the WIDr cell line, the effects of reduced haloperidol and haloperidol were dose-related. By contrast, there was little evidence of a dose effect for (+)-pentazocine and (+)- and (−)-SKF 10,047 in MCF-7, MDA or colon carcinoma cells or in melanoma cells.

The effects on cell proliferation were dependent on the σ ligand and dose used, and on the cell line examined, but a relationship between potency to inhibit cell growth and σ binding affinity was not always apparent for all σ ligands used in every cell line examined. In MCF-7, MDA and colon cells (LIM 1215 and WIDr), (+)- and (−)-SKF 10,047, and (+)-pentazocine had a small effect at 100 μM, producing only a 20–30% inhibition. By contrast, in melanoma cells, (+)- and (−)-SKF 10,047 were completely devoid of any effect at all concentrations used. Furthermore, the reason for the lack of effect of DTG, and the small effect of haloperidol and (+)-pentazocine (20% inhibition at 100 μM), in melanoma cells is uncertain. In melanoma cells, only rimcazole and reduced haloperi-

Table 2
The effect of σ ligands on cell proliferation in diverse human carcinoma cell lines

Cell line	μM	SKF (+)	SKF (−)	DTG	RHAL	HAL	Pentazocine (+)	(−)
MCF-7	100	22 ± 1.4	23 ± 1.6	40 ± 2.3	98 ± 0.5	83 ± 3.5	29 ± 4.1	58 ± 6.1
	50	18 ± 4.4	15 ± 3.2	24 ± 0.5	70 ± 1.2	36 ± 4.3	28 ± 7.7	44 ± 4.5
	25	20 ± 1.7	12 ± 4.3	20 ± 0.5	41 ± 2.5	20 ± 9.4	23 ± 6.6	40 ± 3.4
	12.5	19 ± 1.5	12 ± 2.4	18 ± 4.9	19 ± 1.7	13 ± 3.5	29 ± 4.1	44 ± 4.1
	6.25	19 ± 0.5	6 ± 3.4	12 ± 2.7	14 ± 2.9	7 ± 0.8	14 ± 1.1	25 ± 4.7
MDA	100	12 ± 1.9	21 ± 5.4	37 ± 4.3	72 ± 5.6	34 ± 3.3	25 ± 3.1	55 ± 7.7
	50	3 ± 1.0	8 ± 3.0	24 ± 0	38 ± 4.4	18 ± 4.4	22 ± 2.2	45 ± 5.2
	25	0 ± 0	3 ± 0.9	14 ± 0	23 ± 3.9	8 ± 6.1	19 ± 3.7	30 ± 4.4
	12.5	0 ± 0	2 ± 1.0	11 ± 0	8 ± 5.4	8 ± 2.6	11 ± 5.0	22 ± 5.5
	6.25	0 ± 0	6 ± 0	6 ± 0	0 ± 0	0 ± 0	5 ± 4.1	14 ± 4.3
Melanoma	100	0 ± 0	0 ± 0	0 ± 0	86 ± 1.1	20 ± 1.7	17 ± 0	0 ± 0
	50	0 ± 0	0 ± 0	0 ± 0	24 ± 3.1	14 ± 1.1	15 ± 0	0 ± 0
	25	0 ± 0	0 ± 0	0 ± 0	5 ± 4.0	14 ± 2.2	15 ± 0	0 ± 0
	12.5	0 ± 0	0 ± 0	0 ± 0	8 ± 3.4	12 ± 5.1	18 ± 0	0 ± 0
	6.25	0 ± 0	0 ± 0	0 ± 0	4 ± 2	12 ± 3.3	13 ± 0	0 ± 0
Colon	100	20 ± 3.6	n.d.	47 ± 1.9	63 ± 2.8	32 ± 3.1	22 ± 1.2	17 ± 3.1
LIM	50	13 ± 1.6	n.d.	37 ± 2.2	43 ± 3.5	21 ± 1.6	17 ± 2.6	9 ± 3.7
	25	8 ± 5.2	n.d.	24 ± 3.1	19 ± 4.1	7 ± 2.3	10 ± 1.8	18 ± 5.1
	12.5	7 ± 2.1	n.d.	16 ± 4.4	8 ± 6.0	4 ± 2.9	4 ± 1.2	0 ± 0
	6.25	8 ± 6.0	n.d.	8 ± 4.1	1 ± 0	5 ± 3.1	6 ± 2.0	0 ± 0
Colon	100	33 ± 2.2	36 ± 2.9	51 ± 10	92 ± 1.7	90 ± 0.9	70 ± 1.8	44 ± 1.4
WIDr	50	28 ± 3.4	29 ± 1.2	39 ± 3.1	87 ± 2.1	68 ± 2.9	31 ± 2.2	33 ± 3.8
	25	28 ± 3.8	29 ± 1.2	36 ± 1.7	58 ± 2.0	43 ± 1.7	32 ± 4.1	32 ± 1.1
	12.5	26 ± 4.2	33 ± 3.9	31 ± 2.1	35 ± 0.9	35 ± 1.3	28 ± 3.6	28 ± 3.1
	6.25	25 ± 1.4	29 ± 1.7	34 ± 1.6	33 ± 3.6	37 ± 4.1	29 ± 3.9	26 ± 2.6

n.d. = not done.
Cells were cultured at 2500 cells/well in the presence or absence of σ ligands. After 48 h the cultures were assessed for growth by the MTT assay. Results shown are expressed as percentage inhibition of cell proliferation (mean ± S.E.M., triplicate determinations; $n = 3$) and were normalised to 100% of control cultures without drug.

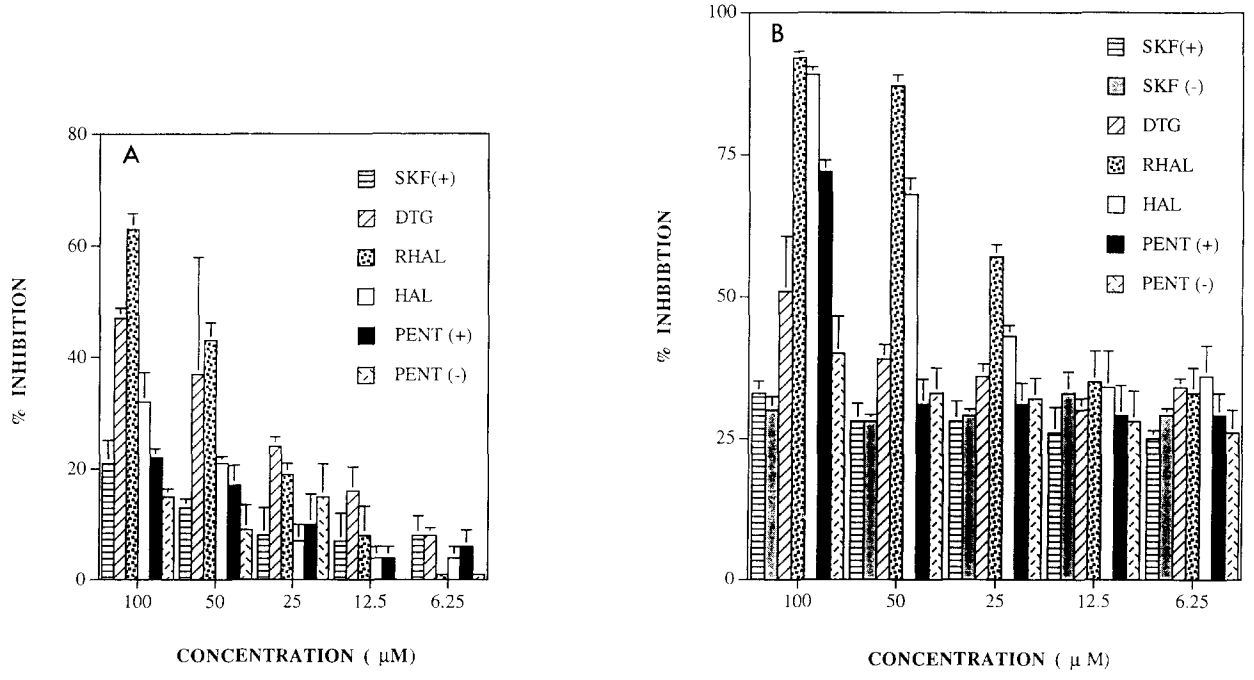

Fig. 4. Growth-inhibitory effect of σ ligands in colon carcinoma cells (A: LIM 1215, B: WIDr). Conditions as for Fig. 2. Results shown (mean ± S.E.M., triplicate determinations; $n = 3$) are normalised to 100% of control cultures without drug.

dol had potent effects on cell proliferation. These results in melanoma cells cannot be explained on the basis of affinities of the ligands for σ sites from previously published binding data, since haloperidol, DTG and (+)-pentazocine all bind with high affinity to σ sites. In one respect, the lack of effect of DTG on melanoma cells in the present study was similar to results obtained in C6 glioma cells by Vilner and Bowen (1993). However, in the latter study (Vilner and Bowen, 1993), (+)-pentazocine was devoid of any effect in C6 glioma cells, whereas in the present study, (+)-pentazocine produced a small effect on melanoma cells.

An interesting aspect of the present studies is that σ ligands for the σ_1 binding site ((+)-SKF 10,047, (+)-pentazocine; see Quirion et al., 1992) consistently produced the smallest effects, whereas ligands which bind to both σ_1 and σ_2 sites, such as haloperidol, reduced haloperidol, DTG and rimcazole, in general produced the largest effects, particularly in the MCF-7 mammary adenocarcinoma cell line. Interestingly, Bowen's group has recently demonstrated that human MCF-7 mammary adenocarcinoma expressed a high density of σ_2 receptors, but showed little or no binding of [^{3}H](+)-pentazocine, suggesting the absence of σ_1 receptors in this cell line (Vilner et al., 1995). By contrast, high densities of both σ_1 and σ_2 receptors were expressed in rodent cell lines (e.g. C6 glioma, N1E-115 neuroblastoma, and NG108-15 neuroblastoma × glioma hybrid), and in human U-138MG glioblastoma, SK-N-SH neuroblastoma, LNCaFGC prostate and ThP-1 leukemia (Vilner et al., 1995). To the extent that σ ligands vary in their affinities for σ_1 and σ_2 sites, the present data may be the result of the activity of σ ligands at multiple sites, since regional variation in the ratio of σ_1 and σ_2 binding in rat brain, liver tissue and cell lines has been demonstrated (Bowen, 1993; Leitner et al., 1994). However, an alternative (and perhaps more likely) possibility could relate to the concentration and/or type of σ receptors present on the various cells. Thus, both σ_1 and σ_2 sites might be contributing to inhibition of cell proliferation in some cell lines, whereas in others such as MCF-7 cells, inhibition of cell proliferation may be mainly via σ_2 sites. This may help account for the apparent inconsistencies in activity of different σ ligands in the cell lines.

The variability of various σ ligands on the proliferative response of tumour cells may also be related to their growth characteristics. Although a detailed analysis of the effects of the σ ligands during growth phases was not undertaken, in general, rapidly growing cells such as MCF-7 and WIDr derived from mucosal tissues such as gut and breast were more sensitive to σ ligands than melanoma cells of comparable growth rate in culture. The reason for this is not clear, but the present study suggests that the doubling time of cell proliferation appears to be a factor influencing the sensitivity of cells to σ ligands. Slow growing colon cancer cells (LIM 1215) with a doubling time of 32 h respond poorly to σ ligands compared to the colon cell line, WIDr, with a doubling time of 15 h. It might be important to note that in vivo, slow growing human tumours were mostly resistant to chemotherapy (DeVita, 1979).

Two limitations should be pointed out in the interpretation of the present data. Firstly, the concentration used to produce the maximum effects (100 μM) is high, although lower doses were also effective over the same time course, similar to results obtained in C6 glioma cells (Vilner and Bowen, 1993; Vilner et al., 1994). In one colon cell line examined for instance (WIDr), the whole range of σ ligands produced 25–40% inhibition at 6.25 μM. However, 100 μM of several non-σ ligands had no effect in MCF-7 and melanoma cells, with mianserin producing an effect at 100 μM only in melanoma cells, and raclopride producing an effect in one of the colon carcinoma cell lines, supporting σ specificity in general. In this regard, the effect of mianserin occurred at the highest concentration used (100 μM), with no effect at lower concentrations, possibly indicating a non-specific effect at this concentration. In addition, the effect of raclopride, a substituted benzamide and selective dopamine D_2 receptor antagonist (Kohler et al., 1985) with no σ activity, occurred in one particular colon carcinoma cell line only (WIDr), and not in the other colon cell line tested (LIM 1215), or in MCF-7 or melanoma cells. Secondly, stereospecific effects between (+)- and (−)-SKF10,047 and (+)- and (−)-pentazocine were weak (i.e. (+) > (−) for σ stereoselectivity), or were in the opposite direction for σ activity (e.g. (−)-pentazocine > (+)-pentazocine in MCF-7 cells). However, the latter data in MCF-7 cells, showing enantioselectivity for (−)-benzomorphans over the corresponding (+)-isomer, are indicative of a σ_2 profile in this cell line, and are consistent with the relative lack of effect of σ_1 receptor ligands to inhibit cell proliferation compared to ligands which bind to σ_2 receptors in the present study. Additionally, several laboratories have failed to observe enantioselectivity for (+)-benzomorphans in binding assays using [^{3}H]DTG (DeHaven-Hudkins and Fleissner, 1992; Karbon et al., 1991) and [^{3}H](+)-3-PPP binding in PC-12 cells (Yang et al., 1989).

The molecular mechanisms whereby σ ligands inhibit cell growth are not known. The morphological alterations produced by σ ligands in the cell lines examined, including cell detachment, rounding of cells and cell death, resemble morphological changes now termed as 'apoptosis' (Arends and Wylie, 1991), a process of active cellular self-destruction in which a series of events are initiated such that the cell partici-

pates in its own death, and suggest that one possible mechanism for these effects of σ ligands on cell proliferation may be induction of apoptosis. In many systems this process is dependent on cations, since the endonuclease required for the intranucleosomal cleavage is calcium and magnesium dependent (Arends and Wylie, 1991). In this respect, studies have demonstrated a relationship between σ binding sites and cations such as Ca^{2+} (Neumaier and Chavkin, 1989; Basile et al., 1992; Ela et al., 1994). Although speculative, it is possible that σ ligands produce their effects on cell growth via modulation of cell Ca^{2+} and/or induction of apoptosis in these cancer cell lines. Studies are presently underway in our laboratory to investigate these possibilities.

In conclusion, high doses of σ-active ligands had profound effects on cell growth in several different human carcinoma cell lines examined, whereas σ-inactive compounds in general did not. Tumour cells derived from different tissues and exhibiting different growth rates had different sensitivity to growth inhibitory effects of σ ligands. These results, and those of others in diverse human tumors (Bem et al., 1991; Barg et al., 1994) and in C6 glioma cells (Vilner and Bowen, 1993; Vilner et al., 1994), suggest that σ binding sites play some role in cell growth and/or proliferation.

Acknowledgements

The authors wish to acknowledge the excellent technical assistance of Maria Ortega and the photography of the Medical Communication Unit, University of Newcastle. This study was supported in part by the National Health and Medical Research Council of Australia to Dr. P.J. Brent. The supply of raclopride from Astra, Sweden is gratefully acknowledged.

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