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σ_2 Receptors regulate changes in sphingolipid levels in breast tumor cells

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Abstract

σ_2 Receptors induce apoptosis in various cell types. The sphingolipid, ceramide as well as the sphingoid bases are involved in cell proliferation. Sphingolipids of MCF-7/Adr- and T47D breast tumor cells were metabolically radiolabeled. The σ_2 receptor agonists (+)-1*R*,5*R*-*E*-8-(3,4-dichlorobenzylidene)-5-(3-hydroxyphenyl)-2-methylmorphan-7-one (CB-184) and 1*S*,2*R*-(−)-*cis*-*N*-[2-(3,4-dichlorophenyl) ethyl]-*N*-methyl-2-(1-pyrrolidinyl)-cyclohexylamine (BD737) caused dose-dependent increases in [^{3}H]ceramide, with concomitant decreases in [^{3}H]sphingomyelin. Both effects were attenuated by the novel σ_2 receptor antagonist, *N*-phenethylpiperidine oxalate (AC927). σ_2 Receptors may produce effects on cell growth and apoptosis by regulating the sphingolipid pathway.

Keywords: σ Receptor; Ceramide; Apoptosis

σ_2 Receptors are highly expressed in a variety of tumor cell lines (Vilner et al., 1995b). We have shown that chronic exposure to σ_2 receptor agonists results in morphological changes and apoptosis in various cell lines, including breast tumor cells, with apoptosis occurring independently of tumor p53 status and independently of known caspases (Vilner et al., 1995a; Crawford and Bowen, 2002). Furthermore, activation of σ_2 receptors in human SK-N-SH neuroblastoma cells results in a D-*myo*-inositol 1,4,5-trisphosphate (IP_3)-independent rise in cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) via rapid and transient release from a thapsigargin-sensitive store in the endoplasmic reticulum (Vilner and Bowen, 2000). This is followed by a latent and sustained release from a thapsigargin-insensitive store, most likely mitochondria. Thus, σ_2 receptors may be involved in regulating cell growth and proliferation.

Sphingolipids play an important role in cell proliferation. Ceramide can cause either increased cell proliferation or growth arrest and apoptosis, depending on the cell type and the downstream signaling pathways affected (Kolesnick and Kronke, 1998). Furthermore, sphingoid bases such as sphingosine-1-phosphate and sphingosylphosphorylcholine also have growth regulating effects, as well as IP_3-dependent and IP_3-independent effects on $[Ca^{2+}]_i$ (Spiegel and Milstien, 2000). Ceramide can be formed by various pathways, including de novo synthesis and hydrolysis of sphingomyelin by sphingomyelinases. Here we investigate the effect of σ_2 receptors on ceramide and sphingomyelin levels in breast tumor cell lines.

Human MCF-7/Adr- and T47D breast tumor cell lines were plated and cultured in 24-well plates as previously described (Crawford and Bowen, 2002). After 24 h, cells were incubated for 48 h with [^{3}H]palmitic acid (20 μCi/ml; specific activity = 30–60 Ci/mmol) and [^{14}C]serine (2 μCi / ml; specific activity = 50–60 mCi/mmol) in serum-free medium in order to label cellular sphingolipids. Cells were treated with or without test compounds for 24 h in serum-free medium. Cells were then washed and scraped from wells, and lipids extracted with chloroform/methanol/0.1 N HCl (4:2:0.5). The organic layer was dried under N_2 and the residue dissolved in 30 μl of chloroform/methanol (1:1). The mixture was spotted onto Silica G-60 plates (EM Science, Gibbstown, NJ), along with authentic samples of sphingomyelin and C_{16}-ceramide (Calbiochem, San Diego, CA) and subjected to thin-layer chromatography. Lipids were first chromatographed in a mobile phase

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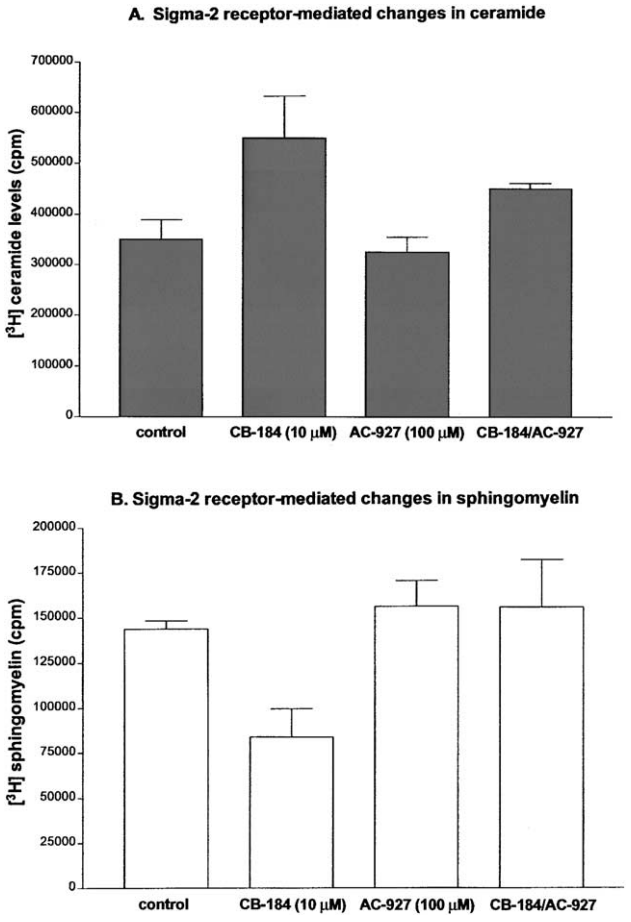

Fig. 1. Chronic exposure of MCF-7/Adr- cells to CB-184 results in an increase in ceramide and a decrease in sphingomyelin levels which are antagonized by AC927. The sphingolipids of MCF-7/Adr- breast tumor cells were metabolically radiolabeled as described in the text. Labeled cells were preincubated in the absence (for no drug controls and cells to be treated with CB-184 alone) or presence of the σ_2 receptor antagonist AC927 (100 μM) in serum-free medium at pH 8.3 for 4 h in order to facilitate access of antagonist to the receptor (Vilner et al., 1999). The medium was then removed and replaced with serum-free medium at normal pH (pH 7.4) containing either no drugs (control), the σ_2 receptor agonist CB-184 (10 μM) alone, AC927 (100 μM) alone, or the combination of CB-184 (10 μM) and AC927 (100 μM), as indicated. Incubation was then carried out for 24 h. Lipids were extracted and samples analyzed by TLC as described in the text. Each bar shown represents the mean and standard error of triplicate samples in one representative experiment. The experiment was replicated three times with similar results, except that the basal radiolabeling of ceramide and sphingomyelin evident in the controls varied across experiments. Panel A: CB-184 (10 μM) produced a significant increase in the levels of [^{3}H]ceramide compared to control (basal) levels ($p < 0.05$, Student's t-test). AC927 (100 μM) had no effect alone, but partially abrogated the effect of CB-184. Panel B: A significant decrease in [^{3}H]sphingomyelin was observed compared to control (basal) levels in response to CB-184 ($p < 0.05$). AC927 had no effect alone, but totally abolished the decrease induced by CB-184.

consisting of chloroform/methanol/water/25% NH_4OH (50:50:2:1) to 70% the height of the plate. The plate was then removed from the chamber, dried and then run in a mobile phase of chloroform/methanol/water/25% NH_4OH (90:10:0.5:0.5) for the entire height of the plate. Sphingolipids were identified by comparison to migration of standards visualized using iodine vapor. Labeled sphingolipids were scraped and quantified by scintillation counting.

(+)-1*R*,5*R*-*E*-8-(3,4-dichlorobenzylidene)-5-(3-hydroxyphenyl)-2-methylmorphan-7-one (CB-184), a selective σ_2 receptor agonist and potent inducer of apoptosis (Bowen et al., 1995; Crawford and Bowen, 2002), caused a dose-dependent increase in [^{3}H]ceramide levels in MCF-7/Adr- cells. The changes in [^{3}H]ceramide for 10 and 100 μM CB-184 were 39.0 ± 0.02 % and 342 ± 0.13 % above basal levels, respectively ($n = 3$ experiments). The CB-184-induced increases in ceramide were accompanied by concomitant decreases in [^{3}H]sphingomyelin levels of 40.0 ± 0.02% and 590 ± 1.1% below basal for 10 and 100 μM CB-184, respectively. Fig. 1 shows that *N*-phenethylpiperidine oxalate (AC927), a novel σ receptor antagonist which attenuates σ_2 receptor-induced cytotoxicity and rises in $[Ca^{2+}]_i$ (Vilner et al., 1999), partially abrogated the CB-184-induced increase in [^{3}H]ceramide and totally abolished the decrease in [^{3}H]sphingomyelin.

Similar results were observed with T47D breast tumor cells, where treatment with CB-184 (100 μM) for 24 h increased [^{3}H]ceramide by 265% above baseline and reduced [^{3}H]sphingomyelin by 87%. 1*S*,2*R*-(−)-*cis*-*N*-[2-(3,4-dichlorophenyl)ethyl]-*N*-methyl-2-(1-pyrrolidinyl)-cyclohexylamine (BD737), which also induces apoptosis and increases $[Ca^{2+}]_i$ (Vilner et al., 1995a; Vilner and Bowen, 2000), increased [^{3}H]ceramide by 610% above baseline in T47D cells during a 24 h treatment at 100 μM.

Several inducers of apoptosis increase ceramide levels, and ceramide-activated protein phosphatase (CAPP) is a known ceramide target (Kolesnick and Kronke, 1998). Interestingly, we have observed that okadaic acid, an inhibitor of CAPP, totally blocked σ_2 receptor agonist-induced cytotoxicity in MCF/Adr- cells, indicating that ceramide and CAPP may be involved in σ_2 receptor-mediated apoptosis (unpublished observation). The current results suggest that σ_2 receptors may utilize sphingolipid products to affect calcium signaling, cell proliferation and survival. The enzymes regulated by σ_2 receptors and the source of ceramide are currently under investigation.

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