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Effects of Selenite on Estrogen Receptor-α Expression and Activity in MCF-7 Breast Cancer Cells

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Abstract To determine whether selenite has estrogen-like activities, the effects of this compound on estrogen receptor-α (ER-α) and other estrogen-regulated genes were measured in the human breast cancer cell line MCF-7. Treatment of cells with 1 uM of sodium selenite resulted in a 40% decrease in the amount of estrogen receptor-α and in a parallel decrease of 40% in ER-α mRNA. Progesterone receptor concentration increased 2.6-fold and pS2 mRNA increased 2.4-fold after selenite treatment. The induction of progesterone receptor and pS2 was blocked by the anti-estrogen ICI-182,780. In transient co-transfection experiments of Wild-type ER-α and an estrogen response element-reporter construct, selenite stimulated CAT activity. In binding assays, selenite blocked the binding of estradiol to ER-α ($K_i = 23 \pm 17$ nM, n = 3) suggesting that this compound interacts with the hormone binding domain of the receptor. To determine whether interaction of selenite with the hormone binding domain results in receptor activation, COS-1 cells were transiently co-transfected with the chimeric receptors GAL-ER, which contains the hormone binding domain of ER-α and the DNA binding domain of the transcription factor GAL4, and a GAL4-responsive CAT reporter gene. Treatment of cells with estradiol or selenite resulted in a three- to five-fold increase in CAT activity. The effects of selenite on the chimeric receptor were blocked by the antiestrogen, suggesting that selenite activates ER-α through an interaction with the hormone binding domain of the receptor. Transfection assays with ER-α mutants identified C381, C447, H524, and N532 as interaction sites of selenite with the hormone binding domain. J. Cell. Biochem. 79: 282–292, 2000.

Key words: ER-α; selenite; estradiol; breast cancer

Breast cancer is the most common malignancy affecting women and is the leading cause of death in women between the ages of 35 and 45 [1993]. Epidemiological studies suggest that endocrine factors play a pivotal role in the etiology of the disease [1993]. The primacy of hormonal factors in the etiology of breast cancer reflects the control of proliferation by estrogens. Because estrogen receptor-alpha (ER-α) is a critical mediator of growth, molecules that can bind to and activate ER-α can potentially increase the risk for breast cancer. A number of natural and man-made chemicals have been identified in the environment which possess estrogenic activity and, therefore, may pose a health risk. This study provides evidence that selenite may be a new candidate nonsteroidal environmental estrogen.

Selenium is an essential micronutrient. It is a component of glutathione peroxidase in eukaryotes and formic dehydrogenase in prokaryotes [Lane, 1989]. It is required for normal growth and development in vivo [Lanfear, 1993] and for the growth of cells in culture [Maurer, 1986]. The most common forms of the element are selenite and selenate, but it is also found in other forms including selenide which is frequently found in food, selenomethionine which is found in plants, and selenocysteine which is found in animals [Combs, 1984]. Human exposure to selenium occurs primarily through ingestion of the organic form, selenomethionine, from cereals, grains, and vegetables [Reddy, 1992]. The recommended dietary intake of selenium is 1 ug/kg body weight

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[Ip, 1991]. Some, but not all, epidemiological studies have suggested that an insufficient intake of selenium may be associated with an increased risk for several kinds of cancers [Reddy, 1992; Medina, 1986]. In the United States, geographic areas with low selenium content in soil appear to have a higher cancer mortality [Vincetti, 1995], however, cancer is not higher in people living in some selenium-deficient areas of the world such as Finland, New Zealand, and China suggesting that other factors influence the disease. A significant inverse association between selenium and lung cancer risk has also been observed [Zheng, 1993; Knecht, 1991]. In animal studies, both a carcinogenic and an anticarcinogenic effect of selenium has been demonstrated [Vincetti, 1995]. At doses higher than the physiological requirement, selenium is a chemopreventive agent [Medina and Shepard, 1980; Medina, 1983; Lane and Medina, 1985; Ip and Sinha, 1981; Thompson, 1982; Thompson and Becci, 1980]. However, at very high doses, selenium is toxic. Occupational exposures to selenium occur primarily in copper refineries, in the production of rectifiers, and in the manufacture of glass, pigments, rubber, and chemicals.

Previous studies from this laboratory have demonstrated that the heavy metal cadmium is a candidate nonsteroidal environmental estrogen. The metal mimics the effects of estradiol in estrogen responsive breast cancer cell lines [Garcia-Morales, 1994] through a high affinity interaction with the hormone binding domain of ER-α [Stoica, 2000]. Cadmium also interacts with the hormone binding domain of the glucocorticoid receptor and blocks dexamethasone binding [Simons, 1990], however, cadmium does not activate the glucocorticoid receptor [Stoica, 2000]. Selenite has also been shown to bind to the hormone binding domain of the glucocorticoid receptor and block dexamethasone binding [Simons, 1990]. To determine whether selenite is a potential environmental estrogen, the effects of selenite on ER-α expression and activity in the ER positive breast cancer cell line MCF-7 were studied. Selenite induced the estrogen-regulated genes progesterone receptor and pS2. The compound appears to activate ER-α through an interaction with the hormone binding domain that also blocked estradiol binding to the receptor. The interaction of selenite with ER-α involves several amino acids in the hormone binding domain suggesting that this compound may form a complex within the hormone binding domain and thereby activate ER-α.

MATERIALS AND METHODS

Tissue Culture

MCF-7 human breast cancer cells were grown in improved minimum essential medium (IMEM) supplemented with 5% fetal calf serum. At 70% confluence, the medium was changed to phenol red-free IMEM supplemented with 5% charcoal-stripped calf serum. Calf serum was pretreated with dextran-coated charcoal to remove endogenous steroids. Cells were maintained in this medium for 2 days prior to treatment and were then treated with sodium selenite, estradiol (Sigma, St. Louis, MO), or the steroidal antiestrogen ICI-182,780 (Zeneca Pharmaceuticals, Wilmington, DE).

Measurement of ER-α and Progesterone Receptor Protein Concentration

Cells were grown as described above. After 24 h treatment with sodium selenite, the cells were washed twice with phosphate-buffered saline and pelleted by centrifugation. Cell pellets were sonicated in a high salt buffer [Saceda, 1988], and the homogenate was incubated on ice for 30 min and centrifuged at 100,000*g* for 1 h at 4°C. Supernatants were assayed for ER-α and progesterone receptor protein. The concentration of ER-α and progesterone receptor protein were determined using specific enzyme immunoassay kits from Abbott Laboratories (North Chicago, IL). Aliquots of the total extracts were analyzed according to the manufacturer's instructions. MCF-7 cells contain variable amounts of estrogen receptor-alpha and -beta. Estrogen receptor-alpha is the predominant isoform of the MCF-7 cell line employed in this study. As measured by an RNase protection assay, approximately 98% of the total ER mRNA is estrogen receptor-alpha.

Measurement of ER-α and pS2 mRNA Amounts

Total cellular RNA was extracted from cells as described previously [Saceda, 1988]. The amounts of ER-α and pS2 mRNA were determined by an RNase protection assay. ^{32}P-Labeled antisense RNA (cRNA) was synthe-

sized in vitro from pOR300 (estrogen receptor) [Saceda, 1988], 36B4 [Saceda, 1988], and pS2 [Masiakowski, 1982] using T7 polymerase. Sixty ug of total RNA were hybridized for 16 h to the ^{32}P-labeled cRNAs. The protected cRNA probes were resolved on 6% polyacrylamide gels. The bands were visualized by autoradiography and quantified by phospho imaging. The amounts of ER-α and pS2 mRNA were normalized using 36B4 as an internal control.

Transient Transfection Assays

A low temperature and low pH calcium phosphate method was employed to transfect COS-1 cells [Chen and Okayama, 1987]. COS-1 cells were plated at a density of 3×10^6 cells/150 mm dish in phenol red-free IMEM containing 10% charcoal-stripped calf serum for 24 h. The cells were transfected with 120 ug of DNA containing 15 ug of an ER-α expression vector (Wild-type or mutant, as described below), 75 ug of the reporter construct pb-CAT (S)MERE, 6 ug of β-galactosidase, and salmon sperm carrier DNA. Sixteen to 18 h after transfection, the precipitate was washed off and the cells were replenished with phenol red-free IMEM containing 10% charcoal-stripped serum in the presence or absence of 1 nM estradiol or 1 uM sodium selenite. The cells were harvested 24 h later and CAT activity was measured as described previously [Garcia-Morales, 1994]. CAT activity was expressed as the percent conversion of chloramphenicol to its acetylated forms and was normalized to the activity of β-galactosidase. The increase in CAT activity in response to treatment was expressed relative to untreated controls. Expression vectors for the Wild-type ER-α and the amino acid mutants (C381A, C417A, C447A, C530A, E523A, D538N, H524A, K529Q, K531Q, and N532D) are described elsewhere [Reese and Katzenellenbogen, 1991; Wrenn and Katzenellenbogen, 1993; Pakdel, 1993; Pakdel and Katzenellenbogen, 1992]. For these transient transfection assays, the estrogen responsive reporter construct pbCAT-(S)MERE was obtained from Dr. D. El Ashry (Lombardi Cancer Center, Georgetown University, Washington DC) [El-Ashry, 1996]. The chimeric receptors GAL-ER and GAL-GR and the reporter plasmid 17M2GCAT are also described elsewhere [Webster, 1988].

Estrogen Receptor-α Binding Assays

The ability of selenite to block estradiol binding to ER-α was determined in cell extracts from MCF-7 cells which were maintained in phenol red-free IMEM containing 5% charcoal-stripped serum. After 2 days in estrogen-depleted medium, the cells were lysed by sonication in a high salt buffer containing 10 mM Tris pH 7.5, 1.5 mM EDTA, 5 mM sodium molybdate, 0.4 M KCl, 1 mM monothioglycerol, 2 mM leupeptin. The homogenate was incubated on ice for 30 min and centrifuged at 100,000*g* for 1 h at 4°C [Stoica, 1997]. The protein concentration of the cell extract was determined by the Bradford method. Cell extracts were preincubated on ice with various concentrations of sodium selenite (1 pM–10 uM). [^{3}H]Estradiol, 10 nM, was then added in the presence and absence of a 200-fold molar excess of diethylstilbestrol (DES) and incubated at 4°C for 16–18 h. Free steroid was removed by the addition of 5% dextran-coated charcoal. The amount of radioactivity was measured by scintillation counting. Specifically bound complexes were calculated by subtracting nonspecific binding from total binding.

RESULTS

Effect of Selenite Treatment on the Concentration of Estrogen Receptor-α Protein

To determine the effect of selenite on the concentration of ER-α protein, an enzyme immunoassay was employed. MCF-7 cells were treated with several concentrations of selenite (0.1–5 uM) for 24 h (Fig. 1). When the cells were treated with 0.1 to 5 uM selenite, a decrease of approximately 40% in estrogen receptor-alpha was obtained. The ER-α concentration decreased from 453 fmol/mg protein in control cells to approximately 270 fmol/mg protein after selenite treatment.

Effect of Selenite on the Steady-State Amount of ER-α mRNA

To determine whether the reduction in ER-α protein paralleled a reduction in the steady-state amount of ER-α mRNA, an RNase protection assay was performed. MCF-7 cells were treated with 0.1, 1, or 5 uM selenite, and the effects of treatment on the steady-state amount of total ER-α mRNA were measured. In these experiments, the amount of ER-α mRNA was

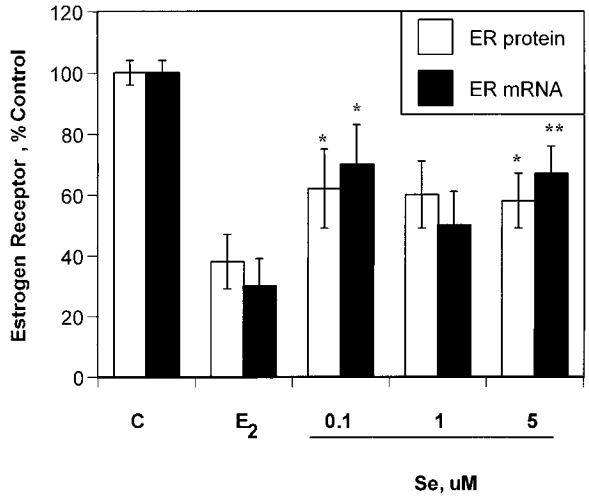

Fig. 1. Effect of selenite on the expression of ER-α protein and mRNA. MCF-7 cells were grown in IMEM medium supplemented with 5% fetal calf serum. At 80% confluence, medium was changed to phenol red-free IMEM and 5% charcoal treated calf serum. Cells were grown in this medium for 2 days and then treated for 24 h with 0.1, 1, or 5 uM sodium selenite or 1 nM estradiol. Estrogen receptor-α protein was determined using an enzyme immunoassay as described under Materials and Methods. Total cellular RNA was extracted and ER-α mRNA was determined by an RNase protection assay as described under Materials and Methods. Autoradiographs were quantified by phospho imaging and the values were represented as the ratio of the ER-α signal to 36B4 signal. Results are presented as percent of control cells and represent the mean value of five to six experiments ± S.D. Statistical differences between treatment with estradiol and selenite were determined using the Student's *t*-test. $*P = 0.01$ to 0.02; $**P = 0.0073$.

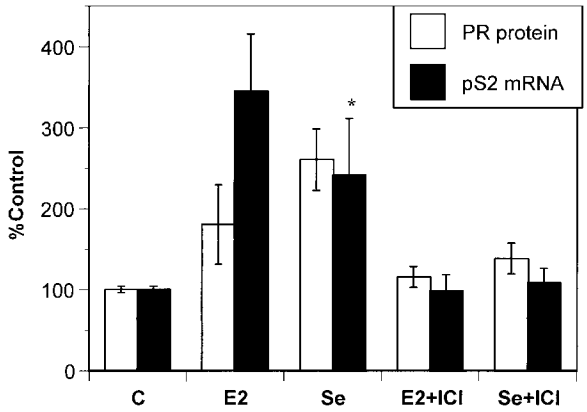

Fig. 2. Effect of selenite on the expression of progesterone receptor and pS2. MCF-7 cells were grown as described in the legend of Figure 1 and treated for 24 h with either 1 uM sodium selenite or 1 nM estradiol in the presence or absence of 500 nM ICI 182,780. Progesterone receptor protein was determined using an enzyme immunoassay as described under Materials and Methods. The amount of pS2 mRNA was determined by an RNase protection assay. Autoradiographs were quantified by phospho imaging and the values were expressed as the ratio of the pS2 signal to 36B4 signal. Results are presented as percent of control cells (mean ± S.D., n = 3 or 4). Statistical differences between treatment with estradiol and selenite were determined using the Student's *t*-test. $*P = 0.0439$.

quantified by phospho imaging, normalized to the amount of 36B4 mRNA and the data are presented in Figure 1 as percent of control of the ratio of the ER-α signal to the 36B4 signal. In this study, treatment with 1 nM estradiol resulted in a 70% decrease in ER-α mRNA amounts, which is in agreement with our previous observations [Saceda, 1988]. Selenite produced an approximately 40% decrease in ER-α mRNA. The decrease in ER-α mRNA following treatment with selenite correlated with the magnitude of the effect on ER-α protein amounts.

Effect of Selenite Treatment on the Concentration of Progesterone Receptor Protein

To determine the effect of selenite on the amount of progesterone receptor, an enzyme immunoassay was performed. MCF-7 cells were treated with 1 uM selenite for 24 h and the concentration of progesterone receptor was measured (Fig. 2). In response to treatment with selenite, the progesterone receptor concentration increased 2.6-fold when compared with control levels. The magnitude of this increase was similar to the increase in progesterone receptor concentration after treatment with 1 nM estradiol. Treatment with 1 nM estradiol resulted in a 1.8-fold increase in progesterone receptor over control values. To determine if the effects of selenite were mediated by ER-α, the ability of the antiestrogen ICI-182,780 (500 nM) to block the effect of selenite was tested. As expected, the antiestrogen blocked the effect of estradiol. The antiestrogen also blocked the effects of selenite suggesting that the effects of this compound are mediated by ER-α.

Effect of Selenite on the Steady-State Amount of pS2 mRNA

To determine whether selenite regulates other estrogen-responsive genes, MCF-7 cells were treated with 1 uM selenite for 24 h and the amount of pS2 mRNA was measured by an RNase protection assay. In this study, selenite

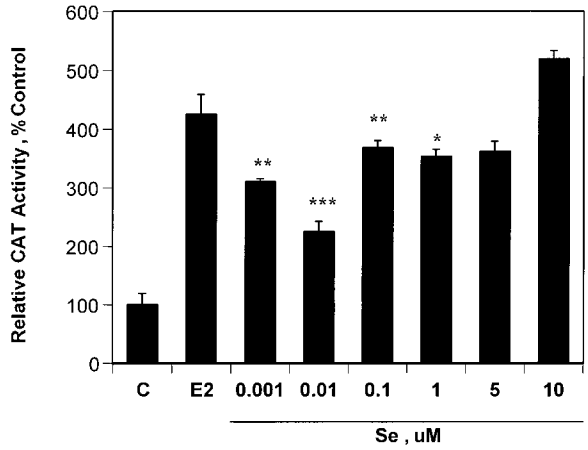

Fig. 3. The ability of selenite to activate ER-α. Wild-type ER-α was transiently co-transfected with an estrogen response element-CAT construct into COS-1 cells. The transfected cells were treated for 24 h with 1 nM estradiol or with concentrations from 1 nM to 10 uM sodium selenite. CAT activity was measured as described under Materials and Methods. The results were normalized to β-galactosidase and were expressed as percent of CAT activity in untreated cells (mean ± S.D., n = 5). Statistical differences between treatment with estradiol and selenite were determined using the Student's *t*-test. $*P$ = 0.0394; $**P$ = 0.001 to 0.002; $***P$ = 0.0005.

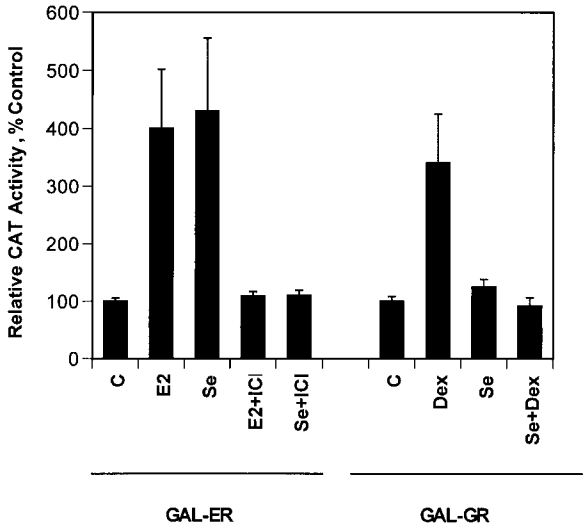

Fig. 4. The ability of selenite to activate GAL-ER. GAL-ER and GAL-GR chimeric genes and a GAL-4-CAT reporter construct were transiently co-transfected into COS-1 cells. The transfected cells were treated for 24 h with 1 nM estradiol, 100 nM dexamethasone, or 1 uM sodium selenite in the presence or absence of 500 nM ICI-182,780. CAT activity was measured as described under Materials and Methods. The results were normalized to the β-galactosidase activity and expressed as percent of CAT activity in untreated cells (mean ± S.D., n = 4).

induced pS2 mRNA by 2.4-fold over control values (Fig. 2). Estradiol, 1 nM, induced a 3.3-fold increase in pS2 mRNA. As in the case of progesterone receptor, the effect of selenite on pS2 mRNA was blocked by 500 nM ICI-182,780 suggesting that the effects of selenite are mediated by ER-α.

Interaction of Selenite With the Hormone Binding Domain of Estrogen Receptor-α

To determine whether selenite activates ER-α, a transient co-transfection assay was employed. A Wild-type ER-α expression vector and an estrogen response element-CAT reporter construct were co-transfected into COS-1 cells. The transfected cells were treated with concentrations of selenite from 1 nM to 10 uM or with 1 nM estradiol. The amount of CAT activity was measured, expressed as percent conversion, and normalized to the amount of β-galactosidase activity (Fig. 3). As expected, estradiol stimulated CAT activity by approximately four-fold. Selenite increased CAT activity by two- to five-fold.

To identify the region of ER-α involved in activation by selenite, chimeric receptors containing the hormone binding domain of either ER-α or the glucocorticoid receptor were employed. These chimeric receptors consist of the DNA binding domain of the yeast transcription factor GAL-4 and the hormone binding domain of either ER-α (GAL-ER) or glucocorticoid receptor (GAL-GR). Stimulation of transcription by GAL-ER or GAL-GR from a GAL-4-responsive CAT reporter gene requires either estradiol or dexamethasone, respectively. When the chimeric receptor GAL-ER and the Gal-4-CAT reporter construct were transiently co-transfected into COS-1 cells and treated with 1 nM estradiol or 1 uM of selenite, there was an approximately four- to five-fold increase in CAT activity (Fig. 4). Addition of the antiestrogen ICI-82,780 (500 nM) blocked the effect of selenite, as well as the effect of the positive control estradiol. Selenite has also been shown to block the binding of dexamethasone to glucocorticoid receptor [Simons, 1990]. Since selenite interacts with the hormone binding domain of the glucocorticoid receptor, GAL-GR was employed as a control and the results are compared in Figure 4. As expected, 100 nM dexamethasone induced an approximately five-fold increase in CAT activity in

cells transfected with GAL-GR. Selenite had no effect and blocked the effect of dexamethasone. Taken together, these results suggest that selenite activates ER-α through an interaction with the hormone binding domain of the receptor. Although selenite has been shown to interact with the hormone binding domain of GR, it does not activate transcription from a reporter gene construct.

Activation of ER-α Mutants by Selenite

Selenite is both capable of forming a coordination complex directly or indirectly with many different amino acids including cysteines. The hormone binding domain of ER-α contains four cysteines at positions C381, C417, C447, and C530. To test the role of these cysteines in the interaction with selenite, each cysteine was mutated to alanine [Reese and Katzenellenbogen, 1991]. The cysteine mutants C381A, C417A, C447A, and C530A, as well as the quadruple mutant C381A C417A C447A C530A were then transiently cotransfected with an estrogen responsive-CAT construct into COS-1 cells and the cells were treated with 1 nM estradiol or 1 uM selenite. The amount of CAT activity was measured, expressed as percent conversion, and normalized to the amount of β-galactosidase activity (Fig. 5a). Following treatment of the cysteine mutants with selenite, there was an approximately four-fold increase in CAT activity with mutants C417A and C530A. In contrast to the effects observed with these mutants, selenite failed to activate the mutants C381A, C447A, and the quadruple mutant C381A C417A C447A C530A suggesting that cysteines C381 and C447 may be involved in activation of ER-α by selenite. To demonstrate that the mutation of cysteine to alanine did not interfere with the activity of the receptor, the transiently transfected cells were treated with 1 nM estradiol. Following hormone treatment, there was an approximate four- to five-fold increase in CAT activity with all the mutants. These results corroborate previous studies employing these mutants which demonstrate that mutation of cysteines in the hormone binding domain to alanine does not alter the ability of estradiol to transactivate the receptor [Reese and Katzenellenbogen, 1991].

Selenite has a negative charge and may interact with histidine and positively charged amino acids. To identify other possible interaction sites within the hormone binding domain of ER-α, histidine H524, lysines K529, K531, and asparagine N532 were mutated [Wrenn and Katzenellenbogen, 1993; Pakdel, 1993; Pakdel and Katzenellenbogen, 1992]. Glutamic acid E523 and aspartic acid D538 were mutated as negative controls. The ability of estradiol and selenite to activate these mutants was also tested in transiently transfected COS-1 cells (Fig. 5B). Selenite did not activate mutants H524A, K529Q K531Q N532D, K529Q K531Q, or N532D, but activated E523Q and D538N resulting in an approximate four-fold increase in CAT activity suggesting that histidine H524, asparagine N532, and at least one of the lysines K529 or K531 may also play a role in the interaction of selenite with ER-α. With the exception of H524, estradiol treatment of all mutants resulted in an approximate four-fold increase in CAT activity suggesting that mutation of these amino acids did not interfere with the activity of ER-α. Estradiol treatment of H524A resulted in 2.8-fold induction of CAT activity, which is in agreement with previously published results [Pakdel, 1993].

Effect of Selenite on the Binding of Estradiol to ER-α

To determine whether selenite blocks estradiol binding to ER-α, the effects of the compound on hormone binding were measured using a single-dose ligand binding assay. Cytosolic extracts from MCF-7 cells were treated on ice with various concentrations of selenite (1 pM–10 nM) for 1 h. The ability of ER-α to bind hormone was then assayed by incubating the extract with 10 nM [^{3}H]estradiol in the presence or absence of a 200-fold molar excess of diethylstilbestrol for 18 h at 4°C. As shown in Figure 6, selenite blocked the binding of estradiol to the receptor. Hormone binding decreased with increasing selenite concentration. The inhibition constant (K_i) for selenite was 23 ± 17 nM, n = 3, as determined by the method of Zhang and Danielsen [1995]. These results demonstrate that selenite blocks the binding of estradiol to ER-α. In contrast to selenite, selenate did not inhibit binding of estradiol to the receptor (data not shown), consistent with the charge differences of these compounds. Similar results were obtained when

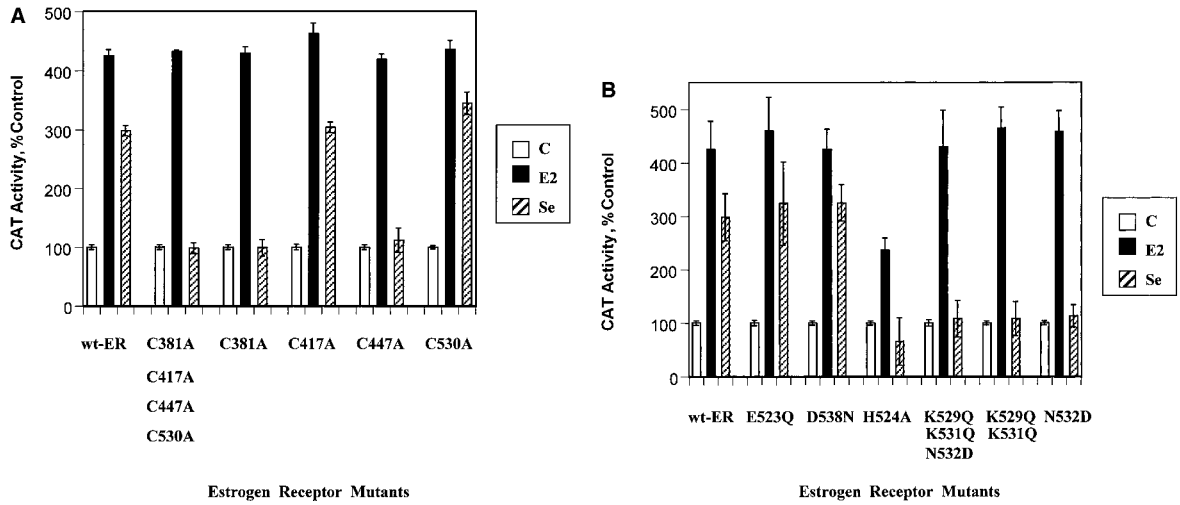

Fig. 5. The ability of selenite to activate Wild-type and mutants of ER-α. **A**: Wild-type ER-α, the cysteine mutants C381A, C417A, C447A, C530A, and the quadruple mutant C381A C417A C447A C530A were transiently co-transfected with an estrogen responsive-CAT construct into COS-1 cells. Transfected cells were treated for 24 h with either 1 nM estradiol or 1 uM of sodium selenite. CAT activity was measured as described under Materials and Methods. The results were normalized to the β-galactosidase activity and were expressed as percent of control of Wild-type ER-α (mean ± S.D., n = 3). **B**: Wild-type ER-α and ER-α mutants E523Q, D538N, H524A, K529Q.K531.Q N532D, K529Q.K531Q, and N532D were transiently co-transfected with an estrogen responsive CAT construct into COS-1 cells and the cells were treated as described above. CAT activity was measured. The results were normalized to the β-galactosidase activity and were expressed as percent of control of Wild-type ER-α (mean ± S.D., n = 3). □ control; ■ estradiol; ▨ selenite.

recombinant human ER-α was used instead of MCF-7 cells (data not shown).

DISCUSSION

In this study, we demonstrate that selenite mimics the effects of estradiol in the ER-α positive human breast cancer cell line MCF-7. Similar to estradiol, selenite decreased the expression of ER-α, increased the expression of progesterone receptor and pS2, and activated ER-α in transient transfection assays. The estrogen-like effects of selenite were inhibited by an antiestrogen suggesting that this compound activate ER-α. The most impressive effect was the ability of selenite to activate ER-α at concentrations as low as 1 nM. High affinity binding to ER-α was demonstrated by the ability of selenite to effectively block estradiol binding (K_i = 23 nM) to the receptor suggesting that this compound is more potent than most known environmental estrogens.

Binding assays and mutational analysis indicate that selenite activates ER-α through the formation of a complex with the hormone binding domain of the receptor. Selenite competes with estradiol for binding to the receptor and activates a chimeric receptor containing the hormone binding domain of ER-α. Mutational analysis identified cysteines C381 and C447, histidine H524, asparagine N532, and at least one, and possibly two, lysines K529 or K531 as potential binding sites consistent with the ability of selenite to interact with amino acids containing a thiol group or a positive charge. The binding of selenite to amino acids in the hormone binding domain may activate ER-α by repositioning the alpha helices similar to the conformational change observed upon hormone binding [Wurta, 1996; Renaud, 1996; Bourguet, 1995; Brzozowski, 1997; Wagner, 1995; Tanenbaum, 1998]. The hormone binding domain of estrogen receptor-alpha contains 12 alpha-helices (H1–H12), folded into a three-layered antiparallel alpha-helical sandwich. The central core layer contains three alpha-helices (H5/6, H9, and H10) sandwiched between two additional layers of helices composed of H1-4, H7, H8, and H11. The central core of the hormone binding domain is flanked by helix H12. Upon binding of the ligand, a conformational change is induced resulting in the formation of a salt bridge between H4 and H12 which repositions helix H12 over the central core and consequently entrapping the hor-

mone in a manner similar to a "mouse trap" [Wurta, 1996]. Ultimately, the repositioning of helix H12 results in the formation of a transcriptionally active receptor. The amino acids, identified as playing a role in the interaction of selenite with ER-α, are located on helices H4, H8, and H11, and in the loop between H11 and H12. Cysteines 381 and 447 are located on helices H4 and H8, respectively. Histidine 524 is located on helix H11 and is in close proximity to estradiol when the ligand is bound to the receptor. Asparagine N532 and lysines K529 and K531 are located in the loop between H11 and H12. It is possible that the interaction of selenite with these amino acids ultimately mimics the effects of estradiol by repositioning H12. However, the precise mechanism by which selenite activates the estrogen receptor remains to be determined.

In previously published studies [Garcia-Morales, 1994; Stoica, 2000], we demonstrated that the heavy metal cadmium also mimics the effects of estradiol in estrogen responsive breast cancer cells by a mechanism similar to the one proposed for selenite. Cadmium appears to activate ER-α through the formation of a high affinity complex with the hormone binding domain of the receptor involving cysteines C381 and C447 and histidine H524. In contrast to the interaction of selenite with positively charged amino acids, cadmium interacts with glutamic acid E523 on helix 11 and aspartic acid D583 at the loop-helix 12 interface which is consistent with the ability of cadmium to form a complex with negatively charged amino acids. The ability of cadmium, as well as selenite, to bind and activate ER-α suggests that these compounds may constitute a new class of nonsteroidal environmental estrogens.

The variation in national breast cancer incidence suggests that environmental factors play an important role in the etiology of the disease. In addition, the observation that the offspring of migrants from areas of low breast cancer incidence to areas of high breast cancer incidence acquire disease rates of the higher area provides further support for a role of the environment in the etiology of the disease. Although the environment appears to be an underlying cause of breast cancer, few environmental risk factors have been identified. The results of this study suggest that selenite is a candidate environmental estrogens and therefore may pose a risk for breast cancer. Although this study suggests that exposure to this compound may pose a risk, most studies to date have not implicated selenium in the etiology of the disease. In fact, it has been suggested that selenium may be an effective chemopreventive agent against several types of cancer. In animal models, selenium reduces the incidence of liver, skin, pancreas, esophagus, colon, and mammary tumors [Ip, 1986; El-Bayoumy, 1994]. Ecological studies also demonstrate an inverse correlation between selenium and most cancers [Shamberger, 1976; Schrauzer, 1977]. Many, but not all, epidemiological studies also show an inverse relationship between selenium and cancers of liver, stomach, colon, pancreas, lungs, bladder, and prostate [Patterson, 1997; Comstock, 1992]. In prevention trials, selenium reduces the incidence of lung, colorectal, and prostate tumors [Clark, 1998; Fleet, 1997]. However, in the case of breast cancer, the role of selenium in the disease is less clear. Although the majority of epidemiological studies show either an inverse correlation or no correlation [Overad, 1991; Hunter, 1990; Van't Veer, 1990; Dorgan, 1998], two studies demonstrate a positive correlation between selenium and breast cancer [Meyer and Verrault, 1987; van Noor, 1993]. A recent prevention trial also demonstrates a small but significant increase in breast cancer in women given selenium [Clark, 1996]. In breast tissue, the selenium content is approximately 1 ug/gm dry weight of tissue and is significantly higher in malignant breast tissue than in normal breast tissue [Garg, 1994; Rizk and Sky-Peck, 1984; Borella, 1997]. Plasma also contains significant amounts of selenium, approximately 100 ng/ml [Overvad, 1991; Gupta, 1994]. Although the precise role of selenium in breast cancer remains to be determined, these studies suggest that the estrogen-like effects of selenium may play a role in the disease.

In summary, this study provides evidence that selenite is a potential environmental estrogen. In estrogen responsive breast cancer cells, the compound mimicked the effects of estradiol resulting in an increase in the steady state levels of progesterone receptor and pS2 and a decrease in the steady state level of

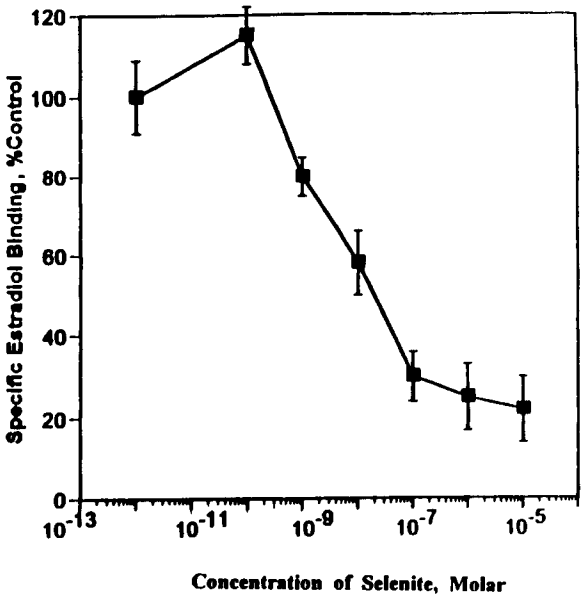

Fig. 6. Effect of selenite concentration on estradiol binding to ER-α. Cytosolic extracts from MCF-7 cells were treated for 1 hour with various concentrations of sodium selenite (1 pM–10 nM). The ability of ER-α to bind hormone was assayed with 10 nM [^{3}H]estradiol in the presence or absence of a 200-fold molar excess of diethylstilbestrol for 18 h at 4°C. The amount of specific binding of [^{3}H]estradiol was determined as described under Materials and Methods and was expressed as percent of control cells (mean ± S.D).

ER-α. The compound appears to activate ER-α through the formation of a high affinity complex with the hormone binding domain of the receptor which blocks the binding of estradiol.

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