2-Methoxyestradiol, an endogenous estrogen metabolite, induces thyroid cell apoptosis

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Abstract

The etiology of autoimmune thyroid diseases is unclear; however, the extreme female predominance suggests that sex hormones may have a pathogenic role. 2-Methoxyestradiol (2-ME) is present in the serum of women during the ovulatory and luteal phases of the menstrual cycle, and during pregnancy. We investigated the actions of 2-ME and estrogen on thyroid follicular cells. 2-ME induced dramatic changes in cell morphology and decreased the viability of the cells, as well as disrupted the structural integrity of cultured thyroid follicles. Flow cytometric analysis showed that 2-ME halted cell proliferation by arresting the cells in the G2/M cell-cycle compartment. Prolonged exposure to 2-ME led to apoptosis and to increased release of the autoantigen thyroid peroxidase (TPO). 17β-estradiol failed to produce a similar effect even in 40-fold molar excess to 2-ME. Co-treatment with estrogen receptor antagonists did not alter the 2-ME effect, indicating that 2-ME was not operating through a classic nuclear estrogen receptor. In conclusion, this study indicates that 2-ME induces G2/M cycle arrest, apoptosis and the disruption of thyroid follicles. This process results in the release of thyroid antigens that may play a role in high incidence of thyroid autoantibodies and autoimmune thyroid disease in women. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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1. Introduction

While the underlying pathogenesis of autoimmune thyroiditis remains unclear, one of the most intriguing features of this disease is the extreme predilection for women (Kloos and Baker, 1996). Autoimmune thyroiditis occurs in at least a 20-fold excess in females (Levine, 1983). The reason for this preponderance is not known, but it is far greater than that for any other autoimmune disease not involving the reproductive organs as a target tissue (Lahita, 1997). There are predisposing conditions for autoimmune thyroiditis including dietary iodine intake and alleles of HLA and CTLA 4 genes (Volpe, 1990; Donner et al., 1997; Lahita, 1997), but these factors are also present in men. Thyroid cells have estrogen receptors and indirect evidence has suggested sex hormone involvement in the pathogenesis of autoimmune thyroiditis (Grossman et al., 1991; Heward and Gough, 1997). However, no direct role for estrogen or its metabolites in thyroid cell function or thyroiditis has been defined.

Apoptosis is a normal physiological process that regulates cell populations and maintains tissue homeostasis (Wyllie et al., 1980). Since apoptosis can lead to target cell destruction, this may result in the release of privileged antigens that can then be presented to the immune system (Ridgway et al., 1994; Albert et al., 1998a). It has been hypothesized that excessive apoptosis mediated by immune cells or by the loss of anti-apoptotic mechanisms in target cells may cause autoimmune diseases (Arends and Wyllie, 1991; Thompson, 1995). There is accumulating evidence that apoptosis is an important mechanism in thyroid cell destruction in thyroiditis (Kotani et al., 1995; Giordano et al., 1997). However, the processes mediating thyroid cell apoptosis are not entirely clear, and may involve both immune and non-immune mechanisms (Arscott and Baker, 1998).

Sex hormones regulate apoptosis in many hormone-responsive tissues. Withdrawal of estrogen or testos-
terone leads to massive apoptosis of breast or prostate cells, respectively (Kyprianou et al., 1990; Detre et al., 1999). Estrogens also have recently been reported to alter the apoptotic potential of hormone-independent cells (Blobel and Orkin, 1996). 2-Methoxyestradiol (2-ME) is an endogenous metabolite of 17β-estradiol and the oral contraceptive agent 17-ethylestradiol. It induces apoptosis in human neuroblastoma cells, bovine endothelial cells and leukemia cells (Nakagawa-Yagi et al., 1996; Yue et al., 1997; Attalla et al., 1998; Tsukamoto et al., 1998). 2-ME is present in blood, urine and most tissues in women and reaches micromolar concentrations during times of high serum estrogen concentration such as pregnancy (Seegers et al., 1997). Despite these findings, the exact physiologic function of 2-ME remains poorly understood (Seegers et al., 1997).

Given the aforementioned information, we considered the possibility that either estrogen or 2-ME might induce cell cycle changes and apoptosis in thyroid cells. To examine this, we evaluated the effects of estrogen and 2-ME on cell cycle progression and the induction of apoptosis in several types of human and rat thyroid cells. The results suggest a potential role for 2-ME in autoimmune thyroiditis.

2. Materials and methods

2.1. Thyroid cell culture

Normal thyroid cells were derived from the contralateral thyroid lobe of a thyroidectomy sample for thyroid carcinoma, and confirmed by pathologic examination. Primary thyroid cells were prepared as previously described (Arscott et al., 1997) and cultured in Cellgro complete media (Mediatech, Herndon, VA) with 10% NuSeur IV (Collaborative Biomedical Products, Bredford, MA) and 10 mM/ml of bovine thyroid-stimulating hormone (TSH; Sigma, St. Louis, MO). The thyroid cells maintained differentiated function as demonstrated by staining for thyroglobulin. To reconstitute follicles, adherent confluent thyrocyte cultures were trypsinized after one passage to make a single cell suspension. After centrifugation and aspiration of the supernatant, the cells were plated at 2 × 10⁶ cells/plate in 10 ml RPMI-1640 supplemented with 10% fetal bovine serum (FBS) in 100 × 15 mm. Cultures were performed in polystyrene Petri plates (Fisher Scientific, Pittsburgh, PA), not treated for tissue culture, that promote follicle formation in the absence of adhesion. The cells subsequently formed lightly adherent round, smooth follicles within 48 h. After 96 h the follicles were gently washed with media and resuspended and re-plated immediately prior to treatments.

FRTL-5 cells were obtained from the American Type Culture Collection (Rockville, MD). The cells were grown in Ham’s F12 medium containing 10% FBS and 15 mM/ml of bovine TSH. The WRO human follicular thyroid carcinoma cell line was a gift from Dr James A. Fagin (University of Cincinnati, Cincinnati, OH). Before initiation of experiments, both the human thyrocytes and FRTL-5 cells were plated overnight in media supplemented with 5% charcoal stripped fetal bovine serum and TSH. The next day, 17β-estradiol, 2-ME, 2-methoxyestriol (Sigma) or vehicle (ethanol, 0.05%) alone were added and cultured with the cells for an additional 2, 4 or 6 days.

2.2. Quantitation of viability and morphologic observation

An MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was used to quantify the viability of cells. Both human thyrocytes and FRTL-5 cells were incubated with concentrations from 0.125 to 100 µM of either 2-ME, 17β-estradiol, 2-methoxyestriol (a closely related analogue of 2-ME) or control vehicle for 2 days before the addition of MTT. The percentage of living cells was defined as absorbance of the treated well/absorbance of the control well × 100% with the OD 595 nm measured by automated plate reader. After 2 days of treatment with 2.5 µM of either 17β-estradiol or 2-ME, the cell morphology was observed and the images were examined by phase-contrast microscopy. The cells were also stained with hematoxylin and eosin and photographed.

2.3. In situ detection of apoptotic cells

Apoptosis in primary human thyrocytes was detected by (terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) staining for fragmented DNA (Arscott et al., 1997). Both adherent and detached populations of cells in culture were pooled and collected by cytopsin. After fixation with 4% of formaldehyde, the cells were analyzed by ApopTag™ staining according to the manufacturer’s protocol (Oncor, Gaithersburg, MD).

2.4. Analysis of cell cycle and quantitation of apoptosis by flow cytometer

Normal human thyrocytes or WRO cells were incubated with 17β-estradiol, 2-ME, or vehicle for 3 days, and then the adherent cells were collected and fixed with 70% of ethanol and stained with propidium iodide (PI). The distribution of cell cycle was determined using a flow cytometer and the data were analyzed by CellQuest software (Becton Dickinson, San Jose, CA). To quantify apoptosis, apoptotic cells were detected by labeling DNA strand breaks with BrdUTP, as previously described (Gorczyca et al., 1993). Briefly, both
and detached cells were fixed with 1% formaldehyde for 15 min on ice and washed with rinsing buffer (phosphate-buffered saline (PBS) supplemented with 0.1% Triton X-100 and 5 mg/ml bovine serum albumin). The cell pellets were incubated for 40 min at 37°C with terminal transferase solution containing: reaction buffer, 10 mM of CoCl₂, 6.25 U of TdT (Boehringer Mannheim, Indianapolis, IN) and BrdUTP (Sigma). The cells then were washed with rinsing buffer and incubated with FITC-conjugated anti-BrdUrd MoAb (Becton Dickinson, San Jose, CA) at room temperature (RT) for 1 h. After incubation, the cells were resuspended in 1 ml of PBS containing PI (5 µg/ml) and RNase (200 µg/ml), and incubated for 30 min at RT in the dark. Green (anti-BrdUrd) and red (PI) fluorescence of cells were determined by flow cytometry.

2.5. ICI-182,780 and tamoxifen treatment

ICI-182,780 is a pure estrogen receptor (ER) antagonist (Wakeling and Bowler, 1992) and tamoxifen is a potent synthetic and therapeutic anti-estrogen agent that can function either as a receptor agonist or antagonist (Jordan and Murphy, 1990). To examine whether 2-ME was affecting cells through interacting with classical ER, thyroid cells were co-treated with 2.5 µM 2-ME, and 100 or 1000 nM of either ICI-182,780 (Tocris Cookson Inc., Ballwin, MO) or tamoxifen (Sigma) for 4 days. The distribution of cells in various portions of the cell cycle was then analyzed by flow cytometry as described above.

2.6. Caspase inhibitor treatment

Caspases are key molecules in apoptosis. In order to study whether apoptosis induced by 2-ME is blocked by caspase inhibitors, we used a generalized caspase inhibitor (z-VAD-fmk) in our experiment. Thyroid cells were pretreated with 20 µM of z-VAD-fmk for 24 h, and then co-treated with 5 µM of 2-ME for 3 days. Apoptotic cells were detected by labeling DNA strand breaks with BrdUTP, as described above.

2.7. Detection of thyroid peroxidase (TPO)

To detect TPO in cell culture supernatants, a sandwich enzyme-linked immunosorbent assay (ELISA) was developed. Briefly, a Maxisorp plate (Nalge Nunc International, Naperville, IL) was coated with M3 ascites (anti-human TPO MoAb developed in our laboratory) diluted 1:100 in coating buffer (50 mM sodium carbonate, 50 mM sodium bicarbonate, pH 9.5) overnight at 4°C, following blocking with 1% dry milk in PBS. Fifty microliters of supernatant was added to each well and the plate was incubated for 1 h at 37°C. After washing with PBS containing 0.05% Tween 20, 100 µl of rabbit anti-human TPO polyclonal antibody, at the concentration 3 µg/ml (designated 56026/97 and developed in our laboratory) was added to each well and the plate was incubated for 30 min at 37°C. The plate was washed and incubated with 100 µl/well anti-rabbit IgG-biotin (Vector, Burlingame, CA) for 30 min at 37°C, then washed again and incubated with 100 µl/well streptavidin-HRP (Sigma, St Louis, MO) for 20 min at 37°C. After washing, 100 µl/well 1-Step™ Turbo TMB-ELISA substrate (Pierce, Rockford, IL) was added and incubated until color was developed. The reaction was stopped with 1 N sulfuric acid and read at a wavelength of 450 nm in an ELISA microtiter reader. Relative units of TPO were calculated as follows:

\[ RU = \frac{(S - B)}{V} \]

where RU, is the relative units of TPO; S, is the absorbance reading of the sample; B, is the background absorbance; and V, is the viability factor, i.e. MTT absorbance of the sample.

2.8. Statistics

Data are expressed as mean ± S.D. and compared by non-paired Student’s t-test (InStat 2.01). A P-value of 0.05 or less was considered significant.

3. Results

3.1. Thyrocyte morphology, viability and follicle structure

Apoptosis has characteristic morphological features including membrane blebbing, rounding up and detachment of cells, and a condensation of the cytoplasm and nucleus. To determine if estrogen or its metabolites can affect thyrocyte viability, we treated cultures with 17β-estradiol, 2-ME, or vehicle as a control. Phase contrast microscopy was used to visualize the FRTL-5 cell morphologic changes induced by these treatments (Fig. 1). In contrast to cells treated with 17β-estradiol which showed normal cell morphology (Fig. 1(a, c)), 2-ME induced changes that included cell spreading and flattening. A significant portion of the cells also became rounded and non-adherent, suggestive of cells undergoing apoptosis (Fig. 1(b)). Hematoxylin and eosin staining documented that cells treated with 2-ME showed evidence of nuclear condensation suggestive of apoptosis (Fig. 1(d)). Similar results were also obtained in human thyrocytes treated with 2-ME (data not shown).

The viability of cells treated with 2-ME was determined by an MTT assay. As shown (Fig. 2), 2-ME at concentrations of 2.5 µM or more caused a significant
loss of cell viability in both human thyrocytes and FRTL-5 cells compared with untreated cells ($P < 0.01$, $n = 6$). However, 17β-estradiol and 2-methoxyestriol did not change the cell viability in either human thyrocytes or FRTL-5 cells, even at molar concentrations that were 40-fold higher (data not shown). Therefore, both the morphologic changes and the inhibitory effect appeared to be specific to 2-ME, and these effects were dose-dependent.

In light of the significant changes in thyroid cell morphology and viability caused by 2-ME, reconstituted thyroid follicles in suspension cultures were used to determine the effects of 2-ME on follicle structure. Follicles treated with 2-ME lost colloid integrity and spread on the surface of the plate (Fig. 3(b)). This was compared to untreated follicles (Fig. 3(a)) that were weakly adherent, spherical in shape and smooth surfaced. Fig. 3(c, d) are lower magnifications of the
untreated and 2-ME treated thyroid follicle cultures, respectively, showing the prevalence of these structural changes. Approximately 80% of the cells were rounded and non-adherent in the 2-ME treated cultures, while few if any cells in control cultures had this appearance.

3.2. Distribution of cell cycle

Untreated primary thyroid follicular cells were stained with PI and the normal cell cycle distribution was determined (Fig. 4(a) left panel). The DNA histogram showed that the majority of cells were in G1 phase with only 9% of cells in the G2/M phase. 17β-estradiol had no effect on the distribution of cells within the cell cycle (Fig. 4(b) left panel). In contrast, 72-h 2-ME treatment significantly enhanced the percentage of cells in the G2/M phase, from the control value of 9–50% (P < 0.01, n = 7; Fig. 4(c) left panel). We also found that colchicine treatment markedly increased the percentage of cells in G2/M phase (data not shown). This indicated that 2-ME affected thyroid cell proliferation by arresting the cells in the G2/M cell-cycle compartment. To further clarify how 2-ME alters the cell cycle, a time course study was performed. G2/M phase arrest appeared to be progressive with 50–60% of either human thyrocytes or FRTL-5 cells arrested after 4 days of treatment, increasing to 88% after 6 days of treatment (data not shown).

In order to investigate whether p53 is involved in 2-ME-induced cell cycle arrest in thyrocytes, we compared the 2-ME effect on normal primary human thyrocytes, which express wild type p53 protein, and WRO cells, which have an inactivating mutation of p53 (Park et al., 1994). As illustrated in Fig. 4(c), both primary human thyrocytes (left panel) and WRO cells (right panel) treated with 2-ME showed an increased percentage of cells in G2/M phase. Therefore, regardless of p53 status, both types of cells had a similar response to 2-ME treatment, with G2/M arrest.

By transfecting FRTL-5 cells with a luciferase reporter construct containing an estrogen response element, we found that 1 μM ICI-187,780 or tamoxifen completely blocked gene induction through endogenous ERs by 17-β estradiol (10 μM). However, treatment with either 1 μM ICI-182,780 or tamoxifen did not prevent the G2/M arrest induced by 2.5 μM 2-ME in either human and rat thyrocytes (data not shown). In addition, WRO cells, which lack estrogen receptors (Weber et al., 1990), respond to 2-ME treatment with G2/M arrest (Fig. 4(c)). This suggested that cell cycle arrest caused by 2-ME was not mediated through the classic estrogen receptors present in thyroid cells.
3.3. Evidence of apoptosis

It has been reported that cell cycle arrest is often associated with the induction of apoptosis (Weber et al., 1990). This was analyzed in the present study through in situ apoptosis detection and flow cytometry.

In situ staining for fragmented DNA showed that 2-ME increased the number of positive stained cells (Fig. 5(b)). To confirm that 2-ME induces apoptosis in thyroid cells, flow cytometric analysis was used to quantify DNA strand breaks. After 6 days of treatment, human thyroid cells were double stained with anti-BrdUrd antibody and PI. As shown in Fig. 5(c), the cells treated with 17β-estradiol have the majority of the population in G1 phase without DNA fragmentation. However, after 2-ME treatment (Fig. 5(d)), most of the cells are arrested in G2/M phase and approximately 30% of cells have DNA fragmentation, reflecting apoptotic cell death. Furthermore, treatment with the caspase inhibitor (z-VAD-fmk) for 3 days decreased the percentage of cells with DNA strand breaks induced by 2-ME, from the value of 16 ± 1.2 to 5.8 ± 0.9 (P < 0.01, n = 4). This indicates the caspase inhibitor blocked 2-ME-induced DNA fragmentation (Fig. 6).

3.4. TPO release from thyrocytes

Further, we study whether the apoptotic cells caused by 2-ME induce the release of TPO. As shown in Fig. 7, normal primary human thyrocytes treated with 2-ME (2.5 μM) for 4 days released more TPO as compared to untreated or estradiol treated cells (P < 0.005, n = 6). This suggests that the increased release of TPO by 2-ME treated cells might facilitate the production of anti-TPO antibodies.

4. Discussion

In the present study, we show for the first time that 2-ME, an endogenous estrogen metabolite, is able to induce apoptosis and follicular disruption in primary human thyrocytes and rat thyroid follicular epithelial cells. The concentrations of 2-ME necessary to cause apoptosis are similar to serum concentrations that occurs during pregnancy, and potentially achievable during regular menstrual cycles (Seegers et al., 1997). These findings are unique in several ways. 2-ME is the first natural compound identified that causes cell-cycle arrest in G2/M phase. While there are drugs that achieve this, the finding of a natural compound with this capability suggests additional physiologic mechanisms controlling the cell cycle. The mechanism of this arrest is also unique. The most prominent mechanism of cell cycle arrest and apoptosis involves p53 protein (Guillouf et al., 1995). However, we found that primary human follicular thyroid cells (wild type p53) and WRO cells (mutant p53) had similar responses to 2-ME. This indicates that 2-ME actions on thyrocytes are independent of functional p53.

Our results concerning 2-ME actions in thyroid cells are in general agreement with prior studies on 2-ME effects in other types of cells (Nakagawa-Yagi et al., 1996; Seegers et al., 1997), but there are a number of significant differences. We observed that 2-ME inhibited thyroid cell proliferation by arresting the cells in the G2/M cell-cycle compartment, and that prolonged exposure to 2-ME led to apoptosis. These findings suggest a correlation may exist between 2-ME induced G2/M arrest and apoptosis in normal thyroid cells. In contrast, similar concentrations of 2-ME cause apoptosis in neuronal cells, but only after pre-treatment by retinoic acid (Nakagawa-Yagi et al., 1996). Also, 2-ME induces apoptosis in transformed skin fibroblast cells, but not in normal fibroblasts (Seegers et al., 1997). Similar degrees of apoptosis also were seen in transformed breast epithelial (MCF-7) cells (Lottering et al.,...
Fig. 5. Induction of apoptosis in primary human thyrocytes by 2-ME. Apoptosis in human thyrocytes was detected by ApopTag staining (a and b) and flow cytometer (c and d). Thyrocytes were treated with vehicle (picture not shown), estradiol (a and c) or 2-ME (b and d) for 6 days. For ApopTag staining, the cells were collected by cytopin and apoptotic cells were detected by ApopTag staining kit. For flow cytometric analysis, the cells were double stained with anti-BrdUrd antibody and PI. Shown are representative data from an individual experiment that was repeated at least twice.

1992) and in lung carcinoma cells (Mukhopadhyay and Roth, 1997). However, the G2/M arrest seen in the thyroid cells is not observed in these cells. 2-ME also had no effect on either cell viability or apoptosis of normal bronchial cells (Mukhopadhyay and Roth, 1998). Thus, thyroid follicular cells are the first normal human primary cells where G2/M arrest and apoptosis with 2-ME are observed.

How 2-ME damage to thyroid cells might promote autoimmune disease is not clear. The pathogenesis of autoimmune diseases is very complex, and appears to involve multiple host and environmental factors. Therefore, it is likely that 2-ME alone is not sufficient to cause disease. However, given the over-representation of autoimmune thyroid disease in females, a finding of hormonally mediated thyroid damage is potentially significant. Based on our in vitro data, 2-ME could result in the increased release of thyroid autoantigens that induces an anti-thyroid immune response. This has been suggested to occur with apoptosis in other disorders, where dendritic cells have been demonstrated to efficiently present antigen derived from apoptotic cells and stimulate class I-restricted CD8 cytotoxic T cells (Thompson, 1995; Albert et al., 1998b). The release of thyroid antigens through thyrocyte apoptosis could lead to a similar type of immune response against the thyroid. Alternatively, the disruption of the thyroid follicular structure by 2-ME could cause leakage of colloid contents and release of thyroglobulin to the immune system without inducing cell death. Disruption of thyroid follicle structure may also lead to a loss of thyrocyte polarity resulting in exposure of normally sequestered thyroid autoantigens, such as TPO, to the vasculature. As this process recurs during pregnancy or monthly during the menstrual cycle, the potential for eliciting an immune response to thyroid antigens would increase, and in a genetically susceptible individual could eventually lead to autoimmune thyroiditis. Therefore 2-ME may serve as a means for thyroid autoantigen exposure to the immune system in females, and this is considered an important, rate-limiting step in the pathogenesis of autoimmune thyroiditis (Weetman and McGregor, 1994).

There are several observations that support this hypothesis. Models of autoimmune disease have indicated that estrogens exacerbate experimental autoimmune thyroiditis (Heward and Gough, 1997), and the amounts employed are high enough to produce significant concentrations of 2-ME. Clinical findings in postpartum thyroiditis, a form of autoimmune thyroiditis,
also support a potential role for 2-ME. This extremely common disorder persists for several months during the post-partum period (Terry and Hague, 1998). While usually self-limited, it can progress to chronic thyroiditis in some individuals (Brown-Martin and Emerson, 1997). Post-partum disease patients demonstrate specific immune responses to the thyroid, most commonly the presence of serum antibodies to TPO (Kuijpens et al., 1998). This type of immune response would be expected from an extensive release of thyroid antigens during pregnancy and an immune response that is then suppressed by the host and resolves after delivery when antigen release subsides. However, in genetically susceptible individuals sensitized by the release of thyroid antigens during pregnancy, subsequent immune-mediated lysis of thyroid cells might perpetuate the sensitized state and result in chronic thyroiditis. Another clinical point supporting this hypothesis is that aside from actual thyroid disease, women also have a far greater frequency of antithyroid antibodies than men and the frequency of these autoantibodies increases with age (Tunbridge et al., 1977; Grossman et al., 1991). This suggests that women are sensitized to thyroid antigens at a far greater frequency than men, and that the development of disease is in part a secondary consequence of this sensitization. Thus, the concept that female sex hormones might lead to an increased sensitization of women to thyroid antigens is supported by clinical findings.

The degree to which 2-ME mediated thyroid cell apoptosis occurs in vivo is not clear, and likely may vary between women. 2-ME is produced by hydroxylation and methylation of 17$\beta$-estradiol, and therefore the concentration of 2-ME is elevated in conditions where 17$\beta$-estradiol is increased, such as pregnancy (Berg et al., 1983). Variations would occur due to the number of times an individual was pregnant or was taking certain oral contraceptives. The source of the 2-ME in the thyroid also is not clear. It is possible that it is mainly derived from plasma since the thyroid is a highly vascular organ. However, the concentration of 2-ME may be higher in the thyroid follicular cells than in plasma, since 2-ME is lipophilic and will accumulate in cell membranes. In addition, thyroid tissue is a potential site for 2-ME synthesis. Catechol-O-methyltransferase, the enzyme that produces 2-ME from 17$\beta$-estradiol, is present in thyroid (Feldman and Wells, 1981; Karhunen et al., 1994) and may locally produce 2-ME. Individual variations in 2-ME synthesis or catabolism therefore could affect the thyroidal concentration of this hormone, and alter an individual’s propensity to thyrocyte destruction. Evaluating the rate of 2-ME synthesis in different individuals and in thyroid tissue may provide insights into individual differences in 2-ME action.

Finally, thyroid cells are known to express a functional estrogen receptor $\alpha$ (Clark et al., 1985) and this suggests that thyrocytes may be sex-hormonally responsive. We have detected both ER$\alpha$ and ER$\beta$ in human thyrocytes by western blot (data not shown). However, the mechanism of 2-ME action is unlikely to involve either classical ER since a forty-fold molar excess of 17$\beta$-estradiol did not induce thyrocyte apoptosis. Further supporting this conclusion were the findings that neither ICI-182,780 nor tamoxifen blocked the induction of cell cycle arrest by 2-ME, and that a thyroid cell line lacking the estrogen receptors responded to 2-ME.
treatment. It is possible that 2-ME signals its actions through either an orphan nuclear receptor or a cell membrane receptor. In either case, the induction of G2/M arrest and apoptosis is highly specific to 2-ME, since this was not produced either by the closely related compound 2-methoxyestriol, progesterone or testosterone (data not shown). Further elucidation of the signal transduction mechanism for 2-ME is crucial to understanding a potential pathophysiologic role for this hormone.

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References


