Modulation of Intestinal Estrogen Receptor by Ovariectomy, Estrogen and Growth Hormone

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Accepted for publication March 31, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

Ovarian hormone deficiency decreases and estrogen (E2) and growth hormone (GH) administrations increase intestinal absorption of calcium (Ca++). However, the underlying mechanisms are uncertain. To examine whether alterations in the binding characteristics of intestinal estrogen receptors (ERs) are involved, we developed and validated methods for simultaneous measurement of intestinal ERs in cytosolic and nuclear fractions and applied these techniques to four groups of female rats: sham-operated, ovariectomized (Ovx), Ovx + 5 μg E2/kg b.wt./day and Ovx + 8 mg GH/kg, b.wt./day. All animals were killed on day 21, and mucosal cells harvested from the duodenum for ER determination. The cytosolic and nuclear ERs were 117.2 ± 2.7 fmol/mg protein and 64.9 ± 1.2 fmol/mg DNA, respectively, in sham-operated rats and decreased by 16.1% and 17.0% to 98.4 ± 1.7 fmol/mg protein and 53.8 ± 1.3 fmol/mg DNA, respectively in Ovx rats (P < .001). E2 therapy prevented completely the decrease in cytosolic and nuclear ERs that occurred in Ovx rat (126.1 ± 2.9 fmol/mg protein and 68.0 ± 3.0 fmol/mg DNA, respectively, in the E2-treated group). Similarly, GH administration prevented the decrease in cytosolic and nuclear ERs that resulted from ovariectomy (119.2 ± 3.2 fmol/mg protein and 63.4 ± 1.3 fmol/mg DNA, respectively, in the GH-treated group). The Kd of nuclear ER-ligand complex was 2.0 ± 0.03 nM in sham-operated rats and was slightly modulated by Ovx, E2 and GH (3.3 ± 0.02, 2.33 ± 0.09 and 2.23 ± 0.04 nM, respectively, P < .001), but the Kd of cytosolic ER-ligand complex was not altered by Ovx, E2 or GH. Our findings indicate that E2 deficiency down-regulates, whereas E2 and GH administrations up-regulate intestinal ERs and prevent ovariectomy-induced decrease in receptor binding affinity. We conclude that E2 deficiency, E2 and GH may modulate intestinal Ca++ absorption, in part, by altering the abundance and binding characteristics of intestinal ERs.

Several laboratories have demonstrated that intestinal Ca++ absorption is low in E2 deficiency and high in E2 replete states (Gallagher et al., 1979, 1980; Gallagher, 1990; Heaney et al., 1978; Francis et al., 1984; Morris et al., 1991; Gennari et al., 1990). These findings suggest that E2 is involved in the regulation of intestinal absorption of Ca++. This view is supported by two recent reports indicating that the epithelial cells of the small intestine are direct targets of E2 action. In 1993, Thomas et al. demonstrated that IEC-6 intestinal crypt cells and segments of the small intestine of rats contain ER mRNA and that IEC-6 cells respond to E2 with increased c-fos mRNA content. In the same year, Arjmandi et al. (1993) demonstrated that intestinal mucosal cells from all segments of the small intestine contain ER immunoreactivity, express the mRNA for ERs and respond directly to 17β-estradiol with enhanced Ca++ transport that is suppressed by transcription and protein synthesis inhibitors. In addition, they reported that in rats E2 promoted intestinal Ca++ absorption without altering plasma levels of 1,25(OH)2 D (Arjmandi et al., 1994), an acknowledged stimulator of intestinal Ca++ absorption. The conclusion from these studies is that intestinal mucosal cells contain functional ERs that respond directly to E2 to promote transcriptional activities and Ca++ absorption. To extend the characterization of the putative intestinal ER and investigate the factors involved in its regulation we have developed techniques for examining intestinal ERs by Scatchard analysis and determined the effects of ovariectomy, E2 and GH administration on their abundance and binding affinity. The hormonal regimens examined were chosen because they are known to alter intestinal Ca++ absorption by mechanisms that are presently unclear. In this study, we explored whether alterations in ER characteristics are components of these mechanisms.

Experimental Procedures

Materials. [3H]E2 (90 Ci/mmol) were obtained from Amersham (Arlington Heights, IL). E2, DES, progesterone, testosterone, DNA, 

ABBREVIATIONS: E2, estrogen, or 17β-estradiol; GH, growth hormone; ER, estrogen receptor; Ovx, ovariectomized; [3H]E2, 17β-[2,4,6,7-3H]estradiol; DES, diethylstilbestrol; HAP, hydroxyapatite; 1,25(OH)2D, 1,25-dihydroxyvitamin D.
sodium azide, EDTA, DTT, NaSCN, HAP and phosphorus assay kit were purchased from Sigma Chemical (St. Louis, MO). Crystalline 1,25(OH)₂D₃ was a gift from Dr. M. Uskokovic of Hoffman La Roche, Inc. (Nutley, NJ). Serum 1,25(OH)₂D₃ assay kit was obtained from Nichols Institute (San Juan Capistrano, CA). Recombinant human GH was provided by Genentech (South San Francisco, CA). The protein assay kit was from BioRad (Hercules, CA).

**Animals and diets.** Female Fisher 344 rats, aged 90 days, were purchased from Harlan Sprague-Dawley (Indianapolis, IN) and used for the experiment described below when they were 95 days old. On arrival at our institution, the rats were housed in a room maintained at 26°C on 14-hr light/10-hr dark cycles. During the experimental period, they were fed Harlan Teklad Laboratory diet (Madison, WI) that contained 0.93% Ca, 0.65% phosphorus and 3.0 IU/g vitamin D and allowed free access to deionized drinking water.

The treatment of animals was in accordance with the guidelines of our Institutional Animal Care and Use Committee and the NIH Guide for the Care and Use of Laboratory Animals.

**Experimental protocol.** At 95 days of age, the rats were divided into four weight-matched groups of 10 animals per group. Group 1 was sham-operated on. Groups 2, 3 and 4 were ovariectomized. Groups 1 and 2 received solvent vehicle daily. Groups 3 and 4 received 5 mg of E₂, and 8 mg GH/kg b.wt./day, respectively. Hormones and solvent vehicle injections were given subcutaneously, beginning from the day after surgery. Group 3 rats received E₂ administration daily for the first 12 days and every other day thereafter. Solvent vehicle was administered to these groups on alternate days after day 12. On day 21, all animals were anesthetized with methoxyflurane and bled from the abdominal aorta. The serum was separated and stored at −20°C for the analysis of Ca⁺⁺, phosphorus and 1,25(OH)₂D concentrations. Duodenum was collected for receptor binding studies. The dose of E₂ was based on what we had found in other studies to be effective in preventing ovariectomy-induced bone loss in rats (Kalu et al., 1991). The dose of GH is in the range that was found to be effective in improving the mechanical strength of graft wounds (Jørgensen et al., 1995) and in increasing cancellous bone volume in Ovx rats (Kalu et al., 1993). Lower doses were less efficacious (Kalu et al., 1991, 1993; Jørgensen et al., 1995).

**Tissue collection and preparation.** Twelve centimeters of small intestine distal to the pylorus, designated the duodenum, was excised, rapidly rinsed with ice-cold physiological saline and slit lengthwise to expose the mucosa. The tissue was placed on a chilled glass plate on an ice bath and mucosal cells scraped from the underlying muscle with a glass slide. Nuclear and cytosolic fractions from the duodenal mucosal cells were prepared essentially as described by Bergman et al. (1987). Briefly, the cells were homogenized in 3 volumes of ice-cold TED buffer (10 nM Tris-HCl, 1 mM EDTA, 1 mM DTT and 0.2 g/liter sodium azide, pH 7.4). The homogenate was placed on a 1.2 M sucrose pad (1:1) in 1.5 ml polypropylene microtube and centrifuged at 6900 × g for 30 min. The supernatant above the sucrose pad was removed and centrifuged at 105,000 × g for 1 hr at 4°C to yield the cytosolic fraction. The sucrose pad was carefully removed, and the nuclear pellet in the bottom was resuspended in a volume of TED buffer equal to the volume of homogenate initially put on the sucrose pad.

**Measurement of cytosolic and nuclear ERs.** Cytosolic and nuclear ERs were measured by an adaptation of the technique of Bergman et al. (1987). Aliquots (300 μl) of cytosolic and nuclear fractions of duodenal mucosal cells were incubated with various concentrations of [³H]E₂ (0.5–8.0 nM) in the presence or absence of 1000-fold excess of unlabeled E₂ for 2 hr at 4°C. At the end of the incubation, 100 μl of 2.5 M NaSCN, dissolved in TED buffer, was added to the reaction mixture to a final concentration of 0.5 M NaSCN to solubilize ER (Sica et al., 1980), and the incubation was continued for 20 hr (nuclear ER) or 40 hr (cytosolic ER). After the incubation period, nuclear samples were centrifuged at 11,800 × g for 1 hr to pellet and remove chromatin (Bergman et al., 1987).

Separation of receptor-bound and free [³H]E₂ was achieved by an adaptation of the HAP procedure (Wecksler and Norman, 1979). Briefly, an equal volume of 50% slurry of HAP, preequilibrated with TED buffer, was added to the tubes with terminal reaction mixture containing [³H]E₂-receptor complex and free [³H]E₂. The tubes were left on ice for 30 min with vortexing every 5 min and were then centrifuged at 2000 × g for 5 min. HAP pellets that contain [³H]E₂-receptor complex were washed three times with 1 ml of TED buffer. Each pellet was then extracted with 1 ml ethanol, and the ethanol extract was transferred to a scintillation vial and evaporated to dryness. To each vial was added 10 ml of scintillation cocktail, and the radioactivity of the samples was counted using a Beckman LS 5000 TD Liquid Scintillation System with an efficiency of 47% for H. Specific binding of [³H]E₂ to ER was calculated by subtracting non-specific binding from total binding in each assay. The equilibrium dissociation constant (Kₐ) was computed by least-squares regression analysis and the number of receptors (Bₘₐₓ) was determined by Scatchard analysis (Scatchard, 1949).

**Specificity analysis of [³H]E₂ binding to intestinal ER.** Aliquots (300 μl) of cytosolic fractions prepared from duodenal mucosal cells were incubated for 16 hr with 1 nM [³H]E₂ in the presence and absence of 500-, 1000- and 2000-fold excess of several unlabelled steroid hormones including DES, E₂, 1,25(OH)₂D, progesterone and testosterone. Bound and free [³H]E₂ were separated by the HAP procedure. Percentage of specifically bound [³H]E₂ was measured by scintillation counting.

**Measurements of serum Ca⁺⁺, phosphorus and 1,25(OH)₂D.** Serum samples were diluted with 0.1% lanthanum solution and analyzed for Ca⁺⁺ by atomic absorption spectrophotometry (model 503, Perkin-Elmer, Norwalk, CT). Serum phosphorus was measured with a Sigma diagnostics phosphorus kit according to the manufacturer’s technique. Serum 1,25(OH)₂D was measured with a radioisotope assay kit obtained from Nichols Institute, and the manufacturer’s protocol was used in carrying out the assay.

**General procedures and statistical analysis.** The protein content of cytosolic fractions was determined by the method of Bradford (1976). DNA content of nuclear preparations was measured by the method of Schneider (1957) using calf thymus DNA as standard. Comparison between treatment groups involved estimation of means, standard errors and analysis of variance (Snedecor and Cochran, 1967). Analysis of variance was performed using a StatView Statistical Package (Abacus Concepts, Berkeley, CA) on a Macintosh Ilsi computer. When the analysis of variance indicated significant difference among mean values, the differences were evaluated by using Fisher’s protected least-significant difference (Fisher’s PLSD) multiple comparison procedure (Fisher, 1935). P ≤ .05 was considered statistically significant.

**Results**

**Effects of ovariectomy, E₂ and GH on body weights, uterine weights and serum parameters.** Initial and final body weights were recorded and the uterus weighed at the termination of the study. The data are shown in table 1. Sham-operated, Ovx, Ovx+E₂ and Ovx+GH-treated rats had similar mean body weights at the start of the study. Ovariectomy significantly increased body weight, and E₂ therapy completely prevented the increase in body weight of Ovx rats. GH administration markedly increased body weights above those of Ovx rats. Ovariectomy caused atrophy of the uterus, as expected. E₂ therapy not only completely prevented the uterine atrophy in Ovx rats but also increased the uterine weights above those sham-operated controls. In contrast, GH administration had no effect on uterine weight. Serum levels of Ca⁺⁺, phosphorus and 1,25(OH)₂D in sham-operated, Ovx, Ovx+E₂ and Ovx+GH-treated rats were measured, and the data are given shown in table 1. Com-
pared with sham-operated animals, the serum level of $\text{Ca}^{2+}$ was not significantly altered by ovariectomy but was increased by E$_2$ and GH administrations. Ovariectomy caused a slight increase in serum phosphorus level, which was prevented by E$_2$ and GH. Ovariectomy had no significant effect on serum 1,25(OH)$_2$D levels, which were 63% and 34% lower in E$_2$- and GH-treated rats, respectively, than in sham-operated rats.

Specificity analysis of [3H]E$_2$ binding to intestinal ER. The specificity of [3H]E$_2$ binding to intestinal ER was determined by competitive binding analysis using several potential steroid competitors of E$_2$ binding to its receptor. That the binding of labeled E$_2$ was specific for estrogenic compounds is demonstrated in figure 1. The figure indicates that only E$_2$ and DES competed effectively with [3H]E$_2$ for binding to duodenal cytosolic ER extracts, whereas progesterone, testosterone and 1,25(OH)$_2$D did not, even at very high concentrations (2000-fold excess).

Characteristics of [3H]E$_2$ binding to duodenal extracts. We established the characteristics of [3H]E$_2$ binding to cytosolic and nuclear ERs by saturation and Scatchard analyses as described in the text. [3H]E$_2$-specific binding data for cytosolic and nuclear ER are shown in figures 2 and 3, respectively. The specific binding of [3H]E$_2$ to duodenal extracts increased with increasing concentrations of [3H]E$_2$ and exhibited a saturation profile for the nuclear fraction at 8 nM [3H]E$_2$, but the saturation profile did not completely plateau at 8 nM [3H]E$_2$ for the cytosolic fraction. Nonspecific binding was <50% of total binding of [3H]E$_2$ at 8 nM for both fractions. Scatchard plots of the specific binding data revealed a single class of binding sites for both cytosolic and nuclear ERs as described in detail later.

Effects of ovariectomy, E$_2$ and GH on the number of duodenal ERs. The number of ER binding sites ($B_{max}$) was quantified by saturation and Scatchard analysis for the dif-

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Ovx</th>
<th>Ovx+E$_2$</th>
<th>Ovx+GH</th>
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<tr>
<td><strong>Body weight (g)</strong></td>
<td></td>
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<tr>
<td>Initial body weight</td>
<td>171.6 ± 2.2</td>
<td>172.6 ± 2.0</td>
<td>172.1 ± 1.7</td>
<td>172.6 ± 1.8</td>
</tr>
<tr>
<td>Final body weight</td>
<td>179.2 ± 2.9</td>
<td>186.7 ± 3.2$^a$</td>
<td>177.2 ± 2.4$^e$</td>
<td>209.8 ± 2.6$^a$</td>
</tr>
<tr>
<td><strong>Uterine weight (g)</strong></td>
<td>0.30 ± 0.01</td>
<td>0.13 ± 0.01$^a$</td>
<td>0.39 ± 0.01$^e$</td>
<td>0.14 ± 0.01$^a$</td>
</tr>
<tr>
<td><strong>Serum calcium (mg/dl)</strong></td>
<td>9.63 ± 0.10</td>
<td>9.40 ± 0.09</td>
<td>10.06 ± 0.16$^a$</td>
<td>10.16 ± 0.18$^a$</td>
</tr>
<tr>
<td><strong>Serum phosphorus (mg/dl)</strong></td>
<td>5.75 ± 0.09</td>
<td>6.08 ± 0.08$^a$</td>
<td>5.61 ± 0.12$^a$</td>
<td>5.71 ± 0.08$^a$</td>
</tr>
<tr>
<td><strong>Serum 1,25(OH)$_2$D (pg/ml)</strong></td>
<td>42.39 ± 3.39</td>
<td>42.49 ± 3.21</td>
<td>15.83 ± 1.67$^d,e,i$</td>
<td>28.08 ± 2.35$^d$</td>
</tr>
</tbody>
</table>

Data are mean ± S.E. for 10 animals per group.

$^a$P < .0001 vs. sham.
$^b$P < .001 vs. sham.
$^c$P < .01 vs. sham.
$^d$P < .05 vs. sham.
$^e$P < .0001 vs. Ovx.
$^f$P < .001 vs. Ovx.
$^g$P < .05 vs. Ovx.
$^h$P < .01 vs. Ovx.
$^i$P < .001 vs. Ovx+GH.

Fig. 1. Specificity analysis of [3H]E$_2$ binding to intestinal ER. Cytosolic fractions prepared from the duodenum were incubated with 10$^{-9}$ M [3H]E$_2$ in the absence of unlabeled hormone (None) or in the presence of (5 × 10$^{-8}$ M) 500- (10$^{-6}$ M) 1000- or (2 × 10$^{-6}$ M) 2000-fold excess of the following unlabeled steroid hormones and related compounds: DES 1,25(OH)$_2$D progesterone (Prog) testosterone (Test) and 17β-estradiol for 16h at 4°C. [3H]E$_2$ bound in the absence of unlabeled hormone was taken as 100% binding. Each bar represents the mean of values from four samples. Each sample consists of a pool of duodenal tissues from two animals. *P < 0.001 vs. None.

Fig. 2. Saturation and Scatchard analyses of [3H]-E$_2$ binding to ERs in duodenal cytosolic fractions.

Fig. 3. Saturation and Scatchard analyses of [3H]-E$_2$ binding to ERs in duodenal nuclear fractions.
have been observed by other investigators (Turner et al., 1982). In contrast, E2 therapy not only completely prevented the decrease in the number of cytosolic and nuclear ERs that occurred in Ovx rats, but E2 also increased the number of cytosolic ERs above those of sham-operated rats (cytosolic ER in Ovx + E2, 126.1 ± 2.0 fmol/mg protein; nuclear ER in Ovx + E2, 68.0 ± 3.0 fmol/mg DNA). Similarly, GH administration restored the decreased number of cytosolic and nuclear ERs that resulted from ovariectomy (from 98.4 ± 1.7 to 119.2 ± 3.2 fmol/mg protein in cytosolic fractions, and from 53.8 ± 1.3 to 63.4 ± 1.3 fmol/mg DNA for nuclear fractions).

Effects of ovariectomy, E2, and GH on the binding affinity to [3H]E2 to duodenal ER. The Kd values of ER-ligand complex for cytosolic and nuclear ERs for the different experimental groups were calculated from Scatchard plots of the binding studies and the data are summarized in figure 7. The Kd for nuclear ER-ligand complex was 2.04 ± 0.03 nM in sham-operated rats and increased significantly by 60% in Ovx rats to 3.26 ± 0.02 nM (P < .001). E2 and GH therapy prevented the increase in Kd that resulted from ovariectomy (2.33 ± 0.09 and 2.23 ± 0.04 nM for E2 and GH, respectively, P = N.S.). In contrast, the Kd of cytosolic ER-ligand complex observed in sham-operated rats was unaltered by ovariectomy, E2 or GH therapy and was higher than that observed for nuclear ERs in sham-operated rats.

Discussion

The effects of ovariectomy, E2, and GH on rat intestinal ERs were investigated in this study. The changes in uterine weights confirmed the success of ovariectomy and E2 therapy. The markedly higher body weights in GH-treated rats attest to the success of the GH treatment. The increase in serum Ca++ we observed in E2-treated rats is consistent with our previous reports (Kalu et al., 1991), and similar results have been observed by other investigators (Turner et al., 1987; Thomas et al., 1988). However, our finding that GH increased serum Ca++ in rats differs from other reports in which GH had no effect on serum Ca++ level in humans (Chipman et al., 1980; Brixen et al., 1995). Ovariectomy is known to alter phosphate homeostasis. A significant reduc-
tion in phosphorus levels has been reported in Ovx rats (Hietala, 1993). In contrast, we observed an increase in phosphorus levels in line with the reports of others that serum phosphate concentration increased after ovariectomy in both rats and humans (Morris et al., 1992; Zofkova et al., 1996). Our finding that $E_2$ depressed ovariectomy-induced increase in serum phosphorus level is consistent with previous report (Turner et al., 1987). GH has been reported to increase serum phosphorus in humans (Chimpan et al., 1980) but not in pigs (Denis et al., 1994). Our data demonstrate that GH also prevented ovariectomy-induced increase in serum phosphorus similar to the effect of $E_2$. Previous reports indicate that the effects of hormones on serum 1,25(OH)₂D levels are complex (Deluca, 1974; Spencer et al., 1981). Our finding that ovariectomy had no effect on serum 1,25(OH)₂D level while $E_2$ administration lowered its concentration is consistent with our previous reports on rats (Kalu et al., 1991). However, there may be species differences as $E_2$ has been reported to increase serum 1,25(OH)₂D levels in humans (Galagher et al., 1980; Van Hoff et al., 1994). It is of note that GH also decreased serum 1,25(OH)₂D levels in this study. While a similar decrease has been observed in humans (Chimpan et al., 1980), GH has also been reported to increase serum 1,25(OH)₂D levels in pigs (Denis et al., 1994) and intact rats (Fleet et al., 1994) in contrast to our findings in Ovx rats. The above observations underline the complexity of the influence of hormones on serum 1,25(OH)₂D levels.

Estrogen receptors are important elements not only in determining the tissues that respond to $E_2$, but also in predicting the responsiveness of target tissues to the hormone. Because this is the first report of in vivo modulation of intestinal ERs by humoral factors, the validity of our findings and conclusions depends, in part, on the reliability of our measurement of the binding characteristics of intestinal ERs. The assay we used is an adaptation of the technique used for saturation and Scatchard analysis of intestinal 1,25(OH)₂D receptors (Wecksler and Norman, 1979; Horst et al., 1990). Our findings indicate that duodenal mucosal cells contain ERs with a single class of binding sites for both cytosolic and nuclear ERs. The saturation analysis data of ER binding showed that the specific binding of $[^3H]E_2$ became saturable at 8 nM $[^3H]E_2$ for the nuclear fraction, but the saturation profile did not plateau completely at 8 nM $[^3H]E_2$ for the cytosolic fraction. Although in preliminary experiments, we increased the concentrations of $[^3H]E_2$ for the cytosolic ER binding above 8 nM, yet the saturation of specific binding in the cytosolic fraction did not occur while nonspecific binding increased to unacceptable levels (data not shown). We are unable to explain why the specific binding of $[^3H]E_2$ for the cytosolic fraction did not become saturable. The binding sites for estrogen interact in a specific fashion with estrogenic compounds such as DES and $E_2$ but have negligible cross-reactivity with other steroids such as testosterone, progesterone and 1,25(OH)₂D. In this study, cold DES and $E_2$ competed effectively and in a dose-dependent manner with $[^3H]$-labeled $E_2$, and at 1000-fold excess concentration permitted only 34% and 13% binding of $[^3H]E_2$ to intestinal ERs, respectively. At the same fold excess concentration, testosterone, progesterone and 1,25(OH)₂D did not compete significantly with $[^3H]E_2$ binding, attesting to the specificity of our ER binding assay.

ERs are widely distributed in mammalian tissues, including the uterus, breast, spleen, blood lymphocytes, kidney, brain, liver (Korach, 1979; Stancel et al., 1973; Osborne et al., 1980; Athreya et al., 1989; Lehrer et al., 1994; Stock et al., 1992; Insel, 1990; Freysschuss et al., 1991) and bone (Komm et al., 1988; Erickson et al., 1988). The presence of functional ERs in intestinal epithelial cells was reported by Thomas et al. (1993). Arjmandi et al. (1993) demonstrated that rat intestinal cell contain ER mRNAs. Later studies from our laboratory further characterized the putative intestinal ERs using RT-PCR analysis, Western blot analysis, Southern blot analysis, ligand binding assay and gel shift assay. It was concluded from these studies that the duodenum contains a variant ER gene that encodes a variant ER protein, and the duodenal ER appears to be a functional variant ER protein of the classic ER (Salih et al., 1996). Our findings from the current study clearly demonstrate that ovariectomy, $E_2$ and GH modulate the abundance and binding characteristics of intestinal ERs. The decrease in cytosolic and nuclear ERs due to ovarian hormone deficiency and its prevention by $E_2$ therapy were unequivocal. In addition, the number of cytosolic ERs rose with $E_2$ therapy to levels above those of sham-operated controls, suggestive but not proof that $E_2$ may have a stimulatory action on duodenal ER synthesis as well. The effects of $E_2$ therapy on $K_d$ are equally of note. While the experimental paradigms did not alter the $K_d$ value for cytosolic ER, ovariectomy increased nuclear $K_d$ by >40% while $E_2$ therapy prevented the increase in nuclear extracts. These findings suggest that $E_2$ regulates not only the number of its intestinal receptors, but the binding affinity of the nuclear receptor to $E_2$. While the effect on binding affinity was unexpected, there is a precedence for homologous regulation of ER by $E_2$. In mammals $E_2$ has been shown to increase the number of uterine ERs, presumably through the stimulation of receptor synthesis at the level of transcription (Bergman et al., 1992).

The other significant observation in this study is the effect of GH on ERs. GH has long been known to stimulate Ca²⁺ absorption, but the action of the hormone is often linked to altered levels of 1,25(OH)₂D in humans (Chimpan et al., 1980) and pigs (Denis et al., 1994). Our current findings that it prevents ovariectomy-induced decrease in intestinal ER complements our recent finding that GH also prevents ovariectomy-induced decrease in intestinal vitamin D receptors (Chen et al., 1997). Our findings indicate that GH has “receptor tropic” effects that are due simply to a generalized anabolic effect of GH on the gastrointestinal tract because receptor numbers were expressed per milligram of cytosolic protein or nuclear DNA. Recent reports indicate that GH can, indeed, induce ER synthesis in primary cultures of hepatocytes by stimulating the transcription of ER mRNA (Jørgensen et al., 1995).

The changes in $E_2$ binding characteristics we observed in ovarian hormone deficiency and $E_2$-treated animals may have important pathophysiological implications. Despite the almost uniform agreement that hypoestrogenic states such as postmenopausal osteoporosis are associated with intestinal Ca²⁺ malabsorption that is corrected by $E_2$ therapy (Gallagher et al., 1979, 1980; Gallagher, 1990; Heaney et al., 1978; Francis et al., 1984; Morris et al., 1991; Gennari et al., 1990), the underlying mechanisms have remained uncertain. Recent observations indicate that Ca²⁺ malabsorption in hypoestrogenic states is related, at least in part, to the abro-
vation of the direct and stimulatory effects of E2 on intestinal Ca\(^{2+}\) absorption. This view is supported by the finding that E2 can stimulate Ca\(^{2+}\) absorption in vivo without altering 1,25(OH\(_2\))D levels (Arjmandi et al., 1994), and it enhances Ca\(^{2+}\) uptake, in vitro, by intestinal mucosal cells (Arjmandi et al., 1993). Our current findings suggest that alterations in the abundance of intestinal ERs may be additional components of the Ca\(^{2+}\) malabsorption that occurs in hypoestrogenic states and that is corrected by E2 therapy. It is of note that nuclear receptor affinity was also decreased by ovariec- tomy and corrected by E2 therapy, which would reinforce the effects of estrogenic state on ER numbers and consequently on intestinal Ca\(^{2+}\) absorption. Other findings suggest that there is also cross-talk between E2 and vitamin D endocrine systems at the level of the intestine, such that E2 deficiency decreases and E2 repletion increases intestinal vitamin D receptors (Chen et al., 1997). In view of the stimulatory effects of E2 and vitamin D on intestinal Ca\(^{2+}\) absorption, the lowering of their intestinal receptors might contribute to the intestinal resistance to the action of 1,25(OH\(_2\))D on Ca\(^{2+}\) absorption in hypoestrogenic states (Francis et al., 1984; Morris et al., 1991; Gennari et al., 1990) and its correction by E2 therapy (Gennari et al., 1990). However, a direct link between ER regulation and Ca\(^{2+}\) absorption remains to be established.

Acknowledgments

We thank Dr. S. Yu for his help, Deanna Hedderich for typing the manuscript and Genentech, Inc., for their generous supply of recombinant human GH.

References


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