Immunolocalization of estrogen receptor α and β in gastric epithelium and enteric neurons

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Abstract

A sexual dimorphism in gastric acid secretion has been known for many years, with women secreting less acid (~40%) than men. The mechanisms mediating this sex difference are unknown, but a role for estrogens is suggested from animal models. Two estrogen receptor (ER) subtypes, ERα and ERβ, mediate genomic effects of estrogens, and mRNA for both subtypes has been detected in the rat stomach. The objective of this study was to determine the cellular distribution of ERα and ERβ proteins in the rat stomach. ERα and ERβ proteins were detected in nuclei of fundic parietal cells and epithelial cells in the progenitor zone. In the antrum, several cells were immunoreactive for ERβ in regions containing stem and neuroendocrine cell types but ERα protein was not detected in antral glands. Both ERα and ERβ proteins were expressed in enteric neurons within the nucleus and cytoplasm, with specific punctate staining for ERα in cell bodies and fibers. These studies are the first to show differences between ERα and ERβ proteins in the epithelial cellular distribution in the fundus and antrum and to detect co-expression in enteric neurons. These results suggest that estrogens may inhibit gastric acid secretion via genomic effects in fundic parietal cells through either ER subtype and in antral neuroendocrine cells via ERβ. Moreover, co-expression of ERα and ERβ in enteric neurons indicates that estrogenic effects could also be mediated through neurogenic reflexes. Our findings imply that direct regulation of multiple cell types by estrogens may contribute to the modulation of gastric functions that have been recognized during the estrous cycle and between the sexes.

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Introduction

Gastric acid secretion has a significant role in the integrated responses to digestion of a meal. A striking sexual dimorphism in gastric acid secretion has been known for many years with women secreting significantly less acid (~40%) than men (Feldman *et al.* 1983, Prewett *et al.* 1991). Reciprocal changes in gastric acid secretion versus serum estradiol concentrations were reported during the menstrual cycle (Sakaguchi *et al.* 1991). In rats, basal gastric acid output is similar in females and males, whereas histamine- and pentagastrin-stimulated gastric acid output can be decreased by 17β-estradiol administration (Omole 1972, Limlomwongse & Piyachaturawat 1982, Aguwa 1984, Adeniyi 1991, Girma *et al.* 1997).

At least two estrogen receptor (ER) subtypes, ERα and ERβ, mediate the genomic actions of estrogens (reviewed in Gustafsson 1999, Muramatsu & Inoue 2000). ERα and ERβ share homologous regions in the DNA and ligand binding domains (~96% and ~58% amino acid homology respectively). These domains confer similar binding affinities for transcriptional activation of estrogen response elements (ERE) and estradiol respectively. However, there are major differences between ERα and ERβ in their tissue distribution, the phenotype of the corresponding knockout mice, and their ligand-dependent transcriptional activities (Barkhem *et al.* 1998). For example, 17β-estradiol can stimulate transcriptional activity at an estrogen response element via either ERα or ERβ, yet can activate an AP-1 element only via ERβ (Paech *et al.* 1997). Tamoxifen and raloxifene, selective estrogen receptor modulators, stimulate AP-1 activity through ERβ, yet do not activate gene transcription via ERα or ERβ at an ERE. These interactions are further compounded by dimerization of ER as either homodimers (α/α, β/β) or heterodimers (α/β) (Cowley *et al.* 1997, Pace *et al.* 1997). Cell-specific gene regulation is also dependent on differential expression of certain ER coactivators and corepressors. Studies in mice with gene deletions for ERα and ERβ have determined that ERα is critical for fertility and bone density in both sexes, whereas ERβ has critical roles in follicular development in females and possibly in prostate and bladder development in males (Korach 2000). These findings underscore the complexity and diversity of estrogenic effects and the mechanisms involved and demonstrate the necessity for knowledge of

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cellular distribution of the ER subtypes in each target tissue.

We reported that ERα and ERβ mRNA were expressed in the epithelium of the rat upper gastrointestinal (GI) tract and that ERβ mRNA was expressed in greater abundance than ERα (Campbell-Thompson 1997). Similar findings were reported in human intestinal tissues, and ERβ mRNA was localized throughout the GI mucosa (Brandenberger *et al.* 1997, Enmark *et al.* 1997). To date, immunolocalization of ERα has been studied in the stomach because ERβ antibodies have only recently become available. However, studies have been conflicting regarding ERα expression, with either low concentrations of ERα protein detected in normal human gastric mucosa (Cameron *et al.* 1992, Ciocca & Vargus Roig 1995) or a lack of expression (Rio & Chambon 1990, Kojima *et al.* 1991).

The specific mechanisms by which estrogens may alter gastric acid secretion are unknown. Direct effects on gastric epithelial cells and secondary neuroendocrine interactions regulate secretion rates. We propose that estrogens acting via nuclear ER directly regulate gastric parietal cell function. The aims of the present study were to determine the cellular distribution of ERα and ERβ genes in the gastric epithelium and to determine the effects of chronic 17β-estradiol administration on gastric acid secretion and gastric ER subtype distribution.

Materials and Methods

Animals and procedures

Young adult female Sprague–Dawley rats (210–270 g, $n=31$, Harlan, Indianapolis, IN, USA) were used. All experiments were approved by the Institutional Animal Care Committee of the University of Florida using procedures of the NIH Guide for the Care and Use of Laboratory Animals. Rats had *ad libitum* access to rat chow and water and were housed individually in wire-bottom cages (14-h light:10-h darkness cycle). Rats were acclimated to conditions for 7 days and divided into groups of ovariectomized and intact females. Bilateral ovariectomy was performed under general anesthesia using sterile techniques. Rats were allowed to recover from surgery for 5 days and then received daily s.c. injections of corn oil vehicle (Ovx) or 17β-estradiol (Ovx-E2; 1, 10, 20, 50 and 100 μg/kg) for 1 week. Ovx and Ovx-E2 rats were randomly assigned into pairs. The daily food consumption of each Ovx-E2 rat was calculated and the same amount of food was given to the Ovx rat. Without pair-feeding, Ovx-E2 rats ($\geq$10 μg/kg) ate 3–8 g food/day less than Ovx controls (data from four pairs monitored for 1 week). Body weights were determined at arrival, surgery and end of the studies. For immunolocalization and *in situ* hybridization studies, Ovx-E2 rats received 20 μg/kg 17β-estradiol.

For serum gastrin analysis, venous blood was obtained by cardiac puncture and stored on ice for 30 min. Samples were spun at 3000 ***g*** and serum collected and stored at −75 °C. Samples were analyzed in triplicate in a single assay using RIA methods established in the laboratory (McGuigan & Wolfe 1982). Human gastrin-17 was used as a standard and results were expressed as pg/ml (sensitivity 10 pg/ml).

Tissue preparation

For immunohistochemistry and *in situ* hybridization, rats were fasted overnight with free access to water and anesthetized with sodium pentobarbital (60 mg/kg i.p.) between 0900 and 1100 h, to avoid diurnal variation. Rats were perfused through the abdominal aorta with Tyrodes's buffer and 2% paraformaldehyde–lysine–periodate fixative (PLP) (McLean & Nakane 1974). Stomach and uterus were dissected and mesentery and fat removed. The stomach was opened along the greater curvature and contents rinsed off with phosphate buffer (PBS, 10 mM KPO_4, 150 mM NaCl, pH 7·4). Tissues were stored in fixative overnight at 4 °C. Full-thickness sections of stomach, including proximal fundus and distal antrum, and uterine horn were embedded in a single paraffin block for each rat. Sections 5 μm thick were placed on Probe-on-Plus slides (Fisher, Pittsburgh, PA, USA) and stored at −20 °C. For RNA analysis, rats were killed and uteri were removed and drained of fluid before freezing and weighing. Uterine total RNA was analyzed as previously reported (Campbell-Thompson 1997).

Antibodies

The specificity of the polyclonal ERα and ERβ antibodies (Table 1) was verified by immunolocalization in the uterus which has been well characterized (Saunders *et al.* 1997, Weihua *et al.* 2000). To detect ERα, we used primarily antisera ER-21 and ER-715, which have no counterparts in ERβ (Kuiper *et al.* 1996), and have been extensively characterized (Table 1). To detect ERβ, we used two antibodies from Affinity BioReagents (Golden, CO, USA) (Table 1). PA1–311 was directed against a synthetic peptide corresponding to the N-terminal amino acids of rat ERβ. This peptide has three of 18 (19%) amino acids in common with rat ERα. PA1–310 was generated against amino acids in the C-terminal region of the rat ERβ and has only 6% amino acid homology with rat ERα. Both ERβ antibodies were affinity-purified by column chromatography and their immunolocalization reported in other tissues (Table 1).

Immunohistochemistry

Tissue sections were heated at 55 °C for 30 min and immediately dewaxed and hydrated using xylenes, graded ethanols, water and PBS. Antigen retrieval methods using

Table 1 Affinity-purified rabbit polyclonal anti-rat ER antibodies used for immunochemistry

	Receptor domain[a]	Amino acids[a]	Source	Immunolocalization in endometrium[b]		Reference
				Nuclear	Cytoplasmic	
Antibody						
ERα						
ER-21	A/B	1–21	Dr G Greene	++++	+	Greene *et al.* 1980 Hess *et al.* 1997
ER-715	D	270–284	NIH[c]	++	−	Furlow *et al.* 1990
MC-20	F	580–599	Santa Cruz[d]	+++	++	
ERβ						
PA1-311	A/B	55–70	Affinity BioReagents	+++	++	Rosenfeld *et al.* 1998
PA1-310	F	467–485	Affinity BioReagents	−	+++	Li *et al.* 1997

[a]ERα and ERβ domains and amino acid numbers from Koike *et al.* (1987) and Kuiper *et al.* (1996) respectively. [b]Immunoreactivity intensity and numbers of positive endometrial epithelial cells grades as: −, no signal; +, barely detectable; ++, minimum; +++, medium; ++++, maximum. [c]NIH/NIDDK Hormone and Pituitary Program, Rockville, MD, USA. [d]Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA.

microwave treatment in citrate buffer and trypsin digestion were also tested with each antibody. For microwave antigen retrieval, sections were immersed in 10 mM citrate solution, pH 6, and heated for four intervals of 2·5 min. The citrate buffer was replenished after each heating. The slides were cooled to room temperature for 20 min and washed in PBS for 5 min. For trypsin digestion, sections were immersed in trypsin solution (0·1 mg/ml trypsin, 200 mM Tris HCl, 4 mM $CaCl_2$, pH 7·7, Sigma Chemical, St Louis, MO, USA) for 12 min at room temperature, followed by washing with PBS containing soybean trypsin inhibitor (10 μg/ml, Sigma) for 5 min. After dehydration or antigen retrieval, sections were incubated with 10% normal donkey serum for 10 min in a humidified chamber, then incubated overnight at 10 °C with antibodies to rat ERα (ER-21, 10 μg/ml) or ERβ (PA1–311, 20 μg/ml) diluted in PBS with 1% BSA. After successive PBS washes (twice, 5 min each), slides were blocked for endogenous peroxidase activity with 3% H_2O_2 in PBS for 10 min and incubated with donkey anti-rabbit peroxidase-conjugated antibodies (1:100, Amersham Life Science, Arlington Heights, IL, USA) for 10 min followed by PBS washes. The sections were reacted with 3,3′-diaminobenzidine tetrahydrochloride (Sigma) and hydrogen peroxide for up to 10 min and washed with distilled water. Sections were lightly counterstained with hematoxylin, dehydrated in graded ethanols through xylene, and mounted. Controls included 1) replacement of the primary antibody with normal rabbit serum or PBS and 2) pre-absorption of the antiserum with peptide antigen using serial sections. Antiserum dilutions were incubated with peptide (1 μg/ml (ER-21) or 20 μg/ml (PA1–311) in PBS, 1% BSA) overnight at 4 °C, followed by centrifugation at 12 000 ***g*** before application to serial sections. Sections were observed with an Axiophot microscope (Carl Zeiss, Oberkochen, Germany) and photographed with 35 mm color slide film (Ektachrome 160T, Kodak). Slides were scanned and compiled using Adobe Photoshop 3·0 before printing.

In situ *hybridization*

Non-radioactive *in situ* hybridization was performed as previously reported (Campbell-Thompson *et al.* 1995). The transcription plasmids contained sequence-verified 334-bp rat ERα (Campbell-Thompson 1997), 349-bp rat ERβ (Campbell-Thompson 1997), and 570-bp rabbit H,K-ATPase (Campbell-Thompson *et al.* 1995) cDNA fragments. A 262-bp rat gastrin cDNA was cloned by RT-PCR from antral total RNA into pCRII vector (Invitrogen, Carlsbad, CA, USA) as reported (Campbell-Thompson 1997). The PCR primer pair included a sense primer (5′-ATGCCT CGACTGTGTG) and antisense primer (5′-CGTATGC TTCCTCTTC) (nucleotides 46–307, Genbank accession M38653). Antisense and sense riboprobes were prepared by *in vitro* transcription using either T7 or SP6 RNA polymerases with digoxigenin-11-UTP (MegaShortScript, Ambion, Austin, TX, USA). Specificity of the cDNA fragments for each gene was evaluated by Northern analysis using total RNA samples from rat uterus or stomach as previously described (Campbell-Thompson 1997).

Gastric and uterine tissue sections were deparaffinized and treated with 0·2 M HCl and proteinase K (1 μg/ml) for 20 min each with washes in wash buffer (TBS; 25 mM Tris–HCl, pH 7·6 and 150 mM NaCl) (Campbell-Thompson *et al.* 1995). Sections were post-fixed in 4% paraformaldehyde and incubated in pre-hybridization buffer for 30 min. Sections were hybridized with 0·1–0·5 ng riboprobe/μl hybridization buffer overnight at 42 °C. Sections were washed in three changes of 2 × saline sodium citrate–50% formamide at 55 °C for 1 h.

Riboprobe hybridization was detected by incubation with sheep anti-digoxigenin antibodies (1:500), followed by an alkaline phosphatase color reaction using nitroblue tetrazolium, bromochloroindoyl phosphate. Levamisole (24 μg/ml) was included to inhibit endogenous alkaline phosphatase activity. Sections were mounted without counterstaining and photographed using black and white print film (T-MAX 100, Kodak). Photographs were scanned and processed as for immunohistochemistry. Control reactions included 1) incubation with hybridization buffer alone, and 2) incubation with a heterologous antisense rat H,K-ATPase β subunit or gastrin riboprobe. Adjacent serial sections were observed for differences in labeling between the antisense ERα and ERβ riboprobes and control conditions.

Stimulated gastric acid secretion

Rats were fasted overnight with access to water and anesthetized with 0·08 ml/100 g body weight of a 1:1 mixture of xylazine (20 mg/ml) and ketamine (100 mg/ml). The pylorus was ligated according to the method of Shay *et al.* (1954) and the abdominal incision closed with suture. Pentagastrin (50 μg/kg i.p.; Ayerst Laboratories, New York, NY, USA) was administered to stimulate gastric acid secretion. Rats were placed in a warm cage and all regained consciousness within 20 min. One hour after pentagastrin treatment, rats were anesthetized with sodium pentobarbital, the stomachs removed and gastric contents collected. Contents were spun at 3000 ***g*** for 10 min. Gastric fluid volume was measured and acid concentration was determined by titration with 0·01 M NaOH to pH 7·0. Acid output was calculated by the product of gastric fluid volume and acid concentration and was normalized to body weight (μEq H^+/h per kg).

Statistical analysis

Data are expressed as means ± S.D. The mean values were analyzed by one-way ANOVA with *post-hoc* Tukey's test. A P value<0·05 was considered significant.

Results

ERα and ERβ distribution in uterus

In this study, ERα was detected in nuclei of the luminal and glandular endometrium and stromal smooth muscle cells of the endometrium and myometrium (not shown). Using PA1–311 for ERβ, a distinct nuclear staining of glandular endometrial cells with slight cytoplasmic staining was observed (not shown). Maximum staining intensity for ERβ was less than for ERα in endometrial cells as reported by others (Weihua *et al.* 2000). ERβ immunoreactivity was also detected in nuclei of vascular smooth muscle cells, but not in stromal smooth muscle cells (not shown). Using PA1–310, ERβ immunoreactivity was detected within the same endometrial cells as with PA1–311, but the immunoreactivity was distributed throughout the cytoplasm, in addition to the nucleus. The intensity of ERα and ERβ immunoreactivity in the epithelial cells of the endometrium was reduced in Ovx-E2 rats compared with Ovx rats, in agreement with the findings of others (Weihua *et al.* 2000). Antigen retrieval procedures did not improve immunoreactivity for these antisera using PLP-fixed tissues and immunoperoxidase detection. Nuclear staining of glandular endometrial cells with ER-21 or ER-715 was essentially eliminated by pre-absorption of the antisera (Fig. 1g, h) and nuclear immunoreactivity was eliminated by absorption of ERβ PA1–311. Cytoplasmic staining with PA1–311 was decreased by ∼50% by peptide absorption. Cytoplasmic staining with PA1–310 was not reduced by peptide absorption (40 μg/ml). Control conditions using substitution of the primary antisera with normal rabbit serum or antibody diluent did not produce cell staining.

Expression of ERα in gastric epithelium

ERα immunoreactivity, detected with ER-21, was localized in fundic epithelial cells within the progenitor zone and in parietal cells throughout the glands (Fig. 1a). Superficial epithelial mucous cells were slightly positive for ERα, whereas chief cells were essentially negative (Fig. 1a). ERα antisera ER-715 showed similar nuclear immunolocalization in the progenitor zone of the fundus, but fewer parietal cells were positive than with ER-21. ERα immunoreactivity was not detected in the antral epithelium (Fig. 1b). Nuclear staining in gastric cells with ERα antisera was eliminated by pre-absorption with the antigenic peptide (not shown).

Expression of ERβ in gastric epithelium

ERβ immunoreactivity, detected with PA1–311, was found in the same fundic epithelial cells as for ERα with both nuclear and cytoplasmic distributions (Fig. 1d). In the antrum, ERβ nuclear immunoreactivity was detected in several cells located in the lower one-third of glands, with fainter staining in the superficial epithelium (Fig. 1e). Cytoplasmic immunoreactivity for ERβ was partially decreased by peptide pre-absorption (∼50%) of the antiserum, suggesting that a portion of the cytoplasmic staining may be non-specific.

Expression of ERα and ERβ proteins in enteric neurons

Enteric neurons in myenteric and submucosal plexi of fundus and antrum were immunopositive for both ERα and ERβ (Fig. 1c, f). Within each plexus, more neurons

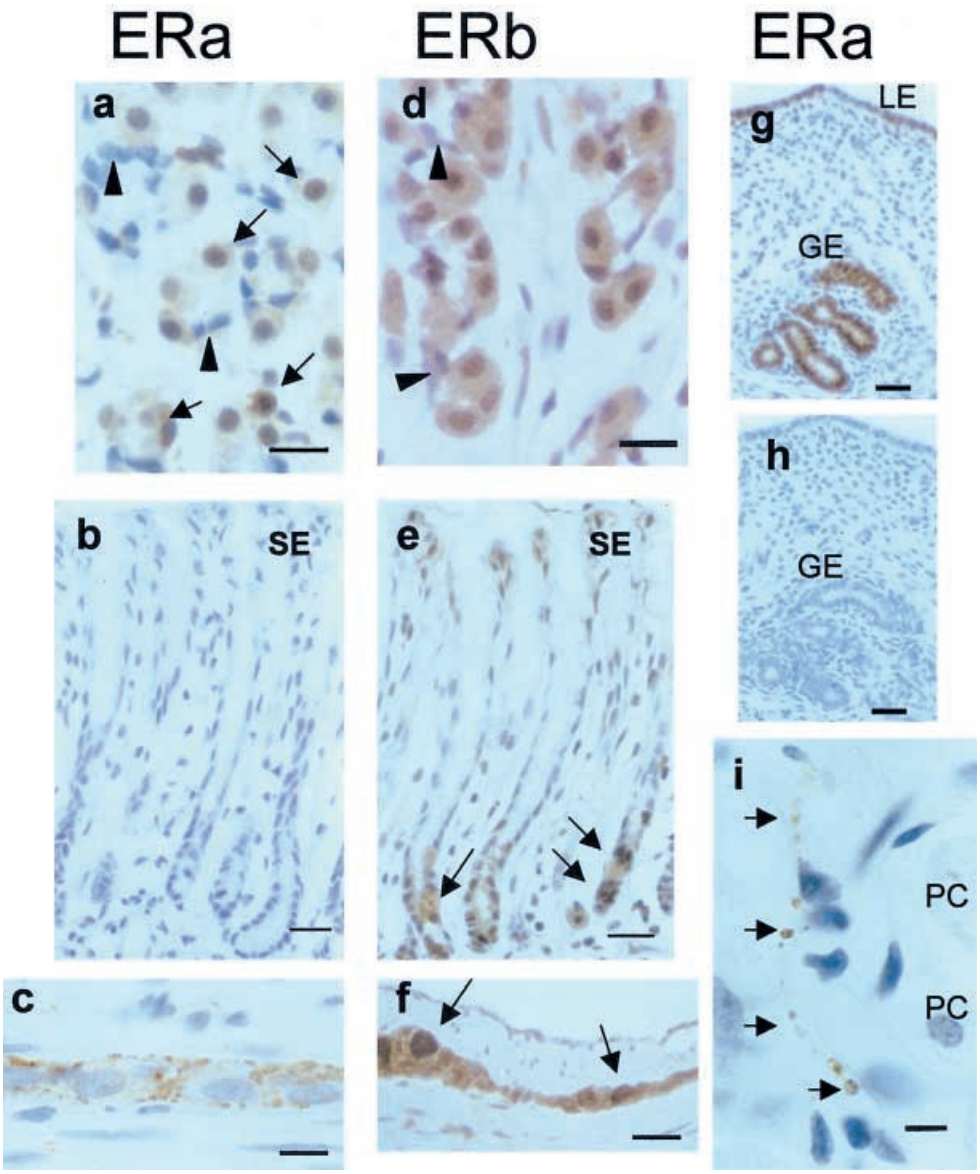

Figure 1 Immunolocalization of ERα and ERβ in the rat stomach. Representative photomicrographs are shown for ERα (a–c, g–i: ER-21) and ERβ (d–f: PA1–311) antisera. In the fundus, ERα (a) and ERβ (d) staining was detected in parietal cells (arrows), but not in chief cells (arrowheads). Cells were not positive for ERα (b) immunoreactivity in the antrum, ERβ (d) was detected in cells located at the base of antral glands (arrows), with fainter staining in the superficial epithelium. Myenteric neurons were stained for ERα (c) in the cytoplasm and for ERβ (f) in nuclei (arrows) and cytoplasm. ERα-positive nerve fibers (i, arrows) were detected near parietal cells at the base of glands. Serial sections of rat uterus show specific ERα (g) protein expression detected using the ER-21 antiserum in glandular and luminal epithelium. This staining was eliminated by peptide pre-absorption (h). LE, luminal endometrium; GE, glandular endometrium; PC, parietal cell; SE, superficial epithelium. Scale bars represent 40 μm (b, e, g, h); 20 μm (a, d); 10 μm (c, f); 5 μm (i).

were positive for ERβ than for ERα. In contrast to the predominantly nuclear localization in endometrium and fundic epithelium, ERα immunoreactivity using ER-21 antisera appeared as punctate foci in the cytoplasm of enteric neurons (Fig. 1c). Specific ERα immunoreactivity could be traced within nerve fibers to submucosal plexi and near parietal cells at the base of gastric glands (Fig. 1i). This punctate staining pattern was detected with both ER-21 and ER-715 antisera and was completely eliminated by peptide pre-absorption. Nuclear and diffuse

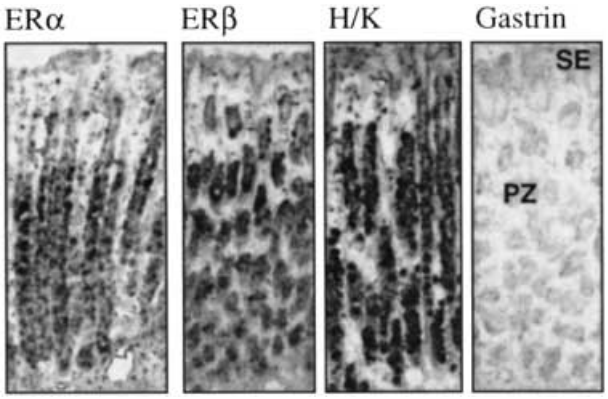

Figure 2 *In situ* hybridization of ERα mRNA in rat fundus. Tissue sections were hybridized with digoxigenin-labeled riboprobes for ERα, ERβ, H/K-ATPase (H/K) and gastrin, as described in Methods. Color reactions were stopped after 15 min for H/K-ATPase and after 60 min for the other probes. ERα and ERβ mRNA were detected in the cytoplasm and peri-nuclear regions of fundic epithelium throughout the progenitor zone and lower glands. H/K-ATPase was detected in parietal cells. A gastrin riboprobe was used as a negative control and showed no staining in epithelium or lamina propria. SE, superficial epithelium; PZ, progenitor zone.

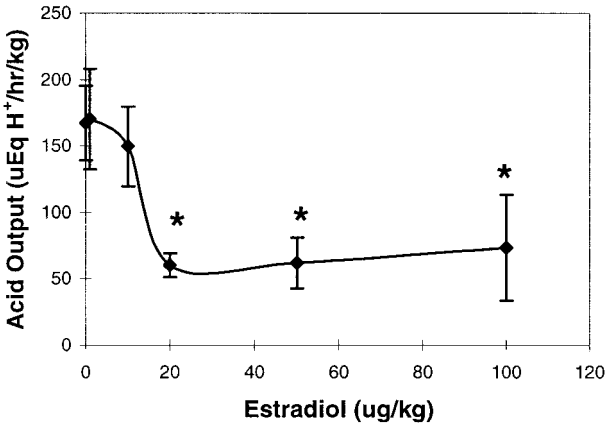

Figure 3 Effects of 17β-estradiol treatment on pentagastrin-stimulated gastric acid output. Gastric contents were collected for 1 h after pentagastrin administration as described in Methods. Dose-dependent decreases in gastric acid output (μEq H^+/h per kg) were observed in Ovx-E2 rats. Values are means ± S.D.; n=4–7 rats/group). *P<0·05 compared with Ovx.

cytoplasmic staining for ERβ was detected in myenteric neurons using PA1–311 (Fig. 1f).

In situ *hybridization for ERα and ERβ mRNA expression in fundic mucosa*

Studies were also performed using *in situ* hybridization to verify cell-specific expression of ERα and ERβ mRNA in fundic glands. Identical cellular distribution of ERα and ERβ mRNA and protein in the rat uterus was observed (not shown). In the fundic epithelium, ERα and ERβ mRNA signals were detected in cells distributed throughout the progenitor zone and lower glands (Fig. 2). Superficial epithelial cells were not positive, in contrast to results for protein expression. The reason for this discrepancy is not known but may indicate cross-reactivity of the antisera with non-ER proteins expressed in the superficial epithelium. A similar pattern was observed using the H/K-ATPase riboprobe, indicating that parietal cells were the predominant cell type that expressed ER.

17β-Estradiol inhibition of gastric acid output and gastrin concentrations

The effects of chronic 17β-estradiol administration (1–100 μg/kg) on gastric acid secretion were studied by means of a standard procedure using pyloric ligation. Treatment with 17β-estradiol significantly inhibited stimulated acid output at doses >10 μg/kg (Fig. 3). Gastric acid output was maximally inhibited to ∼60% of control values. Fasting serum gastrin concentrations were determined and a slight decrease in serum gastrin concentrations was observed after treatment with 100 μg/kg (P=0·62; Table 2). Similar body weights were maintained in Ovx and Ovx-E2 rats by pair-feeding (Table 2). Uterine weights increased ∼2·4-fold in Ovx-E2 rats (Table 2).

Table 2 Effects of 17β-estradiol on body weight, fasting serum gastrin levels, and uterine weights in Ovx rats. Values are means ± S.D. (number of rats)

		17β-Estradiol (μg/kg)	
	Vehicle control	20	100
Body weight (g)	245 ± 12 (21)	231 ± 12 (6)	243 ± 14 (15)
Uterine weight (mg)	235 ± 22 (18)	525 ± 26 (8)*	600 ± 24 (10)*
Serum gastrin (pg/ml)	119 ± 35 (4)	124 ± 11 (6)	86 ± 30 (5)

Female rats were ovariectomized (Ovx) 5 days before receiving daily s.c. injections of 17β-estradiol or vehicle for 7 days. Final body weights and uterine weights are shown for pair-fed rats. Fasting serum samples were analyzed for gastrin concentrations. *P<0·05 compared with Ovx control.

Discussion

Gastric acid secretion by fundic parietal cells is regulated through a complex series of inter-related pathways mediated by paracrine and neuroendocrine mediators and neurotransmitters. Studies in both women and female rat models show that high serum estrogens inhibit gastric acid secretion (Omole 1972, Limlomwongse & Piyachaturawat 1982, Aguwa 1984, Adeniyi 1991, Sakaguchi *et al.* 1991, Girma *et al.* 1997). In the current study, we tested a range of physiological concentrations for 17β-estradiol replacement in Ovx rats and found dose-dependent inhibition up to ∼60% of stimulated gastric acid output after 1 week of chronic 17β-estradiol therapy in pair-fed animals. The demonstration that both ERα and ERβ are expressed in gastric parietal cells suggests that estrogens may directly modulate the secretory activity of these cells. This proposal is supported by *in vitro* studies using isolated parietal cells from either female or male rats, wherein 17β-estradiol treatment (100 nM) decreased histamine-stimulated gastric acid production by ∼20% (Girma *et al.* 1997). Our evidence for the presence of both ERα and ERβ in parietal cells suggests that ER heterodimers could mediate direct estrogenic effects on gastric acid secretion.

Others have reported that 17β-estradiol treatment in Ovx rats suppresses the characteristic ultrastructural changes associated with stimulated acid secretion in parietal cells (Piyachaturawat *et al.* 1983). However, they used extremely high doses of 17β-estradiol (800 μg/kg per day for 7 days). These authors also reported that K^+-ATPase activity was reduced in isolated gastric mucosa obtained from rats treated with this high dose of 17β-estradiol (Limlomwongse & Piyachaturawat 1982). In preliminary studies using our current model, we have been unable to detect differences in either basal or pentagastrin-stimulated parietal cell morphology by transmission electron microscopy between Ovx and Ovx-E2 rats (Campbell-Thompson & Eckard 1996). We also did not detect a difference by immunohistochemistry in gastric ERα and ERβ protein steady-state concentrations, although uterine concentrations of ERα and ERβ protein were regulated inversely with serum estrogen levels. Similarly, one study demonstrated that jejunal ERβ mRNA concentrations were not different between intact and ovariectomized females (Lim *et al.* 1999).

The role of neuroendocrine hormones in the control of gastric acid secretion has been studied for decades. Neuroendocrine hormones, such as gastrin, somatostatin and serotonin, are released in response to feeding and these hormones regulate secretory and motor functions in the stomach. By far the most studies have focused on roles for gastrin and histamine. Gastrin is synthesized in G-cells in antral glands and serum gastrin concentrations are regulated via complex feedback pathways involving other neuroendocrine hormones and neurotransmitters. Feedback stimulation of gastrin production, as reflected in serum gastrin concentrations, is observed after chronic inhibition of gastric acid secretion. Although gastric acid secretion was significantly reduced in Ovx-E2 rats, fasting serum gastrin concentrations were not altered, in agreement with the findings of others (Lichtenberger *et al.* 1976). Other studies from our laboratory have shown that antral gastrin mRNA and protein concentrations are not altered by 17β-estradiol treatment (Campbell-Thompson *et al.* 1999). These results demonstrate that inhibition of stimulated gastric acid output by estradiol does not result from reduced gastrin production.

Gastric enteric neurons and their fibers were also immunoreactive for ERα and ERβ proteins. Specific ERα localization in enteric neurons was detected with two antisera directed to separate domains of ERα. Interestingly, ERα immunoreactivity was detected both in the cell body and within fibers. ERα localization has been described in other cell types in the central nervous system (reviewed in Levin 1999). The ERα antisera (ER-21) used in this study showed nerve fiber staining in the lateral habenula (Wagner *et al.* 1998) and ERβ immunoreactivity (PA1–310) in nerve fibers has been reported in regions of the hippocampus and lateral septum (Li *et al.* 1997). Localization of ER proteins in enteric neurons is provocative in the light of studies showing that estradiol administration delays gastric emptying in Ovx and male rats (Chen *et al.* 1995, Coskun *et al.* 1995). Our results suggest that ERα and ERβ could mediate estrogenic effects on gastric motility through the enteric nervous system.

In conclusion, the results detailed here show that ERα and ERβ proteins are expressed in both gastric epithelial and neuronal cells and administration of physiological concentrations of 17β-estradiol for at least 1 week results in decreased gastric acid output. Our findings indicate that fundic parietal cells and enteric neurons are potential targets for estrogens, and that direct regulation of these cells by estrogens may contribute to the modulation of gastric functions that have been recognized between the sexes and during estrous cycles and pregnancy.

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