The effect of estradiol on the expression of estrogen, progesterone, androgen, and prolactin receptors in human peritoneal fibroblasts

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Abstract

Purpose To assess the ability of fibroblasts isolated from normal peritoneum and adhesion tissues to express various hormone receptors when cultured with exogenous estradiol. **Methods** Primary cultures of fibroblasts from normal human peritoneum and adhesion tissue were treated with zero (control), 10^{-10} , 10^{-8} , and 10^{-6} M concentrations of 17β -estradiol. We performed real time reverse transcriptase polymerase chain reaction to determine mRNA levels of estradiol- α receptor (ER- α) and estradiol- β receptor (ER- β), progesterone receptor (P-R), androgen receptor (A-R), and prolactin receptor (PRL-R) in the two types of fibroblast cultures.

Results In the control groups, P-R and A-R were higher in normal than in adhesion fibroblasts. In adhesion cells, ER- α were higher at 10^{-8} estradiol; ER- β were higher at 10^{-6} M estradiol; P-R remained constant; A-R showed a higher expression at 10^{-10} and 10^{-8} M estradiol; and PRL-R showed an exponential increase at 10^{-10} M estradiol.

Conclusions The inflammatory-like changes manifested by adhesion fibroblasts enhance the anabolic hormones recep-

tor expression (ER- α , ER- β , PRL, and A-R), when exposed to estradiol.

Keywords Adhesions · Ex-vivo study · Fibroblasts · Hormone receptors · Ovulation induction

Introduction

Fibroblasts are the primary cellular component regulating the process of adequate wound healing and scar formation in response to surgery and to pathologic gynecologic processes like endometriosis and pelvic inflammation/infection. How fibroblast functions are altered post-insult, and to what extent this ‘activation’ persists, is not well understood. Fibroblast activation after pelvic surgery and the expression of sex hormone receptors in adhesion tissue was the object of an immunohistologic study by Wiczysk et al. [1]. The authors found that adhesion tissue cells (mesothelial, smooth muscle, and fibroblast) manifested estrogen and progesterone receptors and growth factors in various concentrations. They concluded that sex hormones may be important in the genesis of the permanent fibrovascular bands between pelvic organs.

In this pilot study, we used fibroblasts as a model to verify whether exposure to high estradiol levels would influence their response to express hormone receptors. Indeed, if we consider that in clinical practice, in infertile patients with endometriosis, infertility treatments are started soon after the surgical resection of the lesions (if no other medical therapies are undertaken), knowing the effects of such therapies on adhesion formation could help in the immediate post-operative management of the patients, given the related pathology of small bowel obstruction and/or long term abdominal–pelvic pain. In fact, adhesions

Capsule When adhesion, but not normal, fibroblasts are exposed to increasing estradiol concentrations, they show enhanced expression of anabolic hormone receptors (ER- α , ER- β , PRL, and A-R).

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are believed to contribute to infertility in 40% of infertile couples [2]. This pathologic tissue could be even more adversely affected by high estradiol concentrations.

In a previous study by Saed et al., the investigators were able to establish and characterize fibroblast cultures from normal peritoneum and adhesion tissue from the same patients [3]. This accomplishment allowed identification of different behaviors of the resting versus activated fibroblasts in different oxygen concentration environments.

In this prospective experimental study we wanted to verify whether adhesion fibroblasts would modify their response to different estradiol concentrations in expressing a range of hormone receptors.

Materials and methods

Source and culture of human fibroblasts

Normal parietal peritoneal tissue from the anterior abdominal wall lateral to midline incision and adhesion tissue were excised from three patients undergoing laparotomy for pelvic pain, at the initiation of the surgery following entry into the abdominal cavity. Normal peritoneum was at minimum three inches from any adhesions. Subjects did not have an active pelvic or abdominal infection and were not pregnant. All patients gave informed written consent to tissue collection, which was conducted under a protocol approved by the Wayne State University Institutional Review Board.

Harvested tissue samples were immediately placed in standard media (Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal bovine serum, 2% penicillin and streptomycin). Tissues were cut into small pieces in sterile culture dish and transferred into another fresh T-25 flask with 3 ml of dispase solution (2.4 U/ml, GIBCO BRL, Life Technologies, Inc.). The flasks were incubated overnight at 37°C in an environ-shaker (LAB-LINE Instruments, Inc.). Samples were then centrifuged for 5 min at 1,400 g, transferred into a fresh T-25 flask with pre-warmed DMEM medium and put in 37°C incubator (95% air and 5% CO₂), and outgrowth of fibroblasts generally took 2 weeks. When confluence was reached the cells were transferred to 90-mm tissue culture dishes and cultured in standard media with 10% fetal bovine serum (FBS). Thereafter, the confluent dishes were subcultured by trypsinization (1:3 split ratio). Studies were conducted using passage 3–5 cells to maintain comparability.

As previously reported, normal peritoneal fibroblasts were morphologically similar to the adhesion tissue fibroblasts, but the two cell populations had been previously histochemically characterized [3].

Cell treatment

Each of the fibroblast populations were separated into four different dishes and treated with increasing molar concentrations (0, 10^{-10} , 10^{-8} , and 10^{-6} M) of 17 β -estradiol (Sigma-RBI, St. Louis, MO) in ethanol and incubated for 24 h at 37°C. This would mimic the passage from a physiologic to a high estradiol concentration environment as clinically seen in ovulation induction for infertility treatments. The amount of estradiol contained in FBS is minimal, and would not significantly change the total amount of estradiol to which the two cell subcultures were equally exposed to [4]. The increasing molar concentrations corresponded to 27, 2,724, and 272,380 pg/mL, respectively, based on the formula for conversion of moles/L to gram/L concentration of estradiol (the molecular weight of 17 β -estradiol is 272.38 D; a 1 M solution of estradiol is equal to 272.38 g estradiol in 1 L of solvent). The 10^{-10} M estradiol concentration approximately corresponds to the estradiol level in early follicular phase of a human physiologic menstrual cycle, and the 10^{-8} increase tested in this study mimics the estradiol concentrations reached during a human ovulation induction cycle with gonadotropins, while the 10^{-6} represents a pharmacologic concentration that closely approximates levels seen in the ovarian follicular fluid during ovulation induction. The same volume of ethanol used to dilute estradiol was added to the culture media of the controls for consistency. Total RNA was isolated from normal peritoneal and adhesion fibroblasts with the use of the monophasic solution of phenol and guanidine isothiocyanate/Trizol method as previously described [5].

Preparation of complimentary DNA

A 20 μ l reaction volume, including 1 μ g total RNA, 1 μ l oligo (dT) (500 μ g/ml) (Invitrogen, CA) and 1 μ l 10 mM dNTP Mix (Invitrogen, CA) was heated to 65°C for 5 min and then quickly chilled on ice. A master mixture containing 4 μ l 5 $\times$ First Strand Buffer, 2 μ l 0.1 M DTT, 1 μ l RNaseOut Recombinant Ribonuclease Inhibitor (40 units/ μ l) (Invitrogen, CA) was added, and incubated at 42°C for 2 min. One microliter (200 units) of SuperScript II (GIBCO BRL) was added to each reaction and incubated for 50 min at 42°C, to inactivate the enzyme by heating at 70°C for 15 min.

Oligonucleotide primers for real-time reverse transcriptase polymerase chain reaction (RT-PCR) amplification of reverse-transcribed complimentary DNA (cDNA) were selected with the aid of software program Oligo 4.0 (National Bioscience, Inc., Plymouth, MN). Sequences of the oligonucleotides used for amplification

of estradiol- α receptor (ER- α), estradiol- β receptor (ER- β), progesterone receptor (P-R), androgen receptor (A-R), prolactin receptor (PRL-R), and β -actin mRNA are shown in Table 1.

Quantitative reverse transcriptase polymerase chain reaction analysis

Quantitative RT-PCR was performed using a QuantiTect SYBR Green RT-PCR kit (Qiagen) and a Cepheid 1.2 f Detection System. RT-PCR was performed in a 25 μ l total reaction volume including 12.5 μ l of 2 $\times$ QuantiTect SYBR Green RT-PCR master mix, 3 μ l of cDNA template, and 0.2 μ M each of target specific primers designed to amplify a part of each gene. To quantify each target transcript, a standard curve was constructed with serial dilutions of standard plasmid (Invitrogen). Amplification was performed as follows: 95°C for 10 min, and 40 cycles of 95°C for 15 s and different annealing temperature (55–60°C). After PCR, a melting curve analysis was performed to demonstrate the specificity of the PCR product as a single peak. A control, containing all the reaction components except for the template, was included in all experiments. The amount of each mRNA was then normalized to a housekeeping gene, β -actin. The PCR reaction conditions were programmed for each primer as follows: an initial cycle was performed at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min and then a final cycle at 72°C for 7 min to allow completion of product synthesis.

Our main outcome measure was the quantification of mRNA levels (mRNA copies/ μ g RNA) of Estradiol- α , Estradiol- β , progesterone, androgen, and prolactin receptors in both adhesion tissue and normal peritoneum fibroblasts. We used *T*-test (SPSS for Windows, version 13.0; SPSS Inc., Chicago, IL) to compare the mean receptor mRNA values obtained in the two cell cultures (normal and adhesion fibroblasts). A *p*-value lower than 0.05 was considered statistically significant.

Results

Each fibroblast population in the normal and adhesion groups showed a uniform response to the administration of exogenous estradiol.

In the control cultures, without estradiol added, both normal and adhesion fibroblasts expressed steroid and protein receptors (Fig. 1a). In particular, normal fibroblasts showed a significantly higher receptor expression for estradiol- α ($4,042 \pm 684$ mRNA copies/ μ g RNA in normal versus $2,614 \pm 227$ copies/ μ g RNA in adhesion fibroblasts, respectively; $p < 0.03$), prolactin ($13,544 \pm 551$ mRNA copies/ μ g RNA in normal versus $11,811 \pm 78$ copies/ μ g RNA in adhesion fibroblasts, respectively; $p < 0.007$), progesterone ($87,074 \pm 1,425$ mRNA copies/ μ g RNA in normal versus $33,955 \pm 1,588$ copies/ μ g RNA in adhesion fibroblasts, respectively; $p < 0.001$), and androgen ($32,841 \pm 2,667$ in normal versus $5,735 \pm 285$ copies/ μ g RNA in adhesion fibroblasts, respectively; $p < 0.001$) receptors than their counterpart adhesion fibroblasts. Expression of estradiol- β ($112,266 \pm 19,724$ mRNA copies/ μ gRNA in normal versus $112,101 \pm 8,606$ copies/ μ g RNA in adhesion fibroblasts, respectively; ns) was no different in the two cell cultures.

In the cultures exposed to 10^{-10} M estradiol concentration (which simulates the estradiol level in the early follicular phase of a human physiologic menstrual cycle) (Fig. 1b), estradiol- α receptor expression was comparable in the two cell cultures ($3,894 \pm 886$ mRNA copies/ μ g RNA in normal versus $4,747 \pm 1,763$ copies/ μ g RNA in adhesion fibroblasts, respectively; ns). Estradiol- β receptor expression was higher in normal as opposed to adhesion fibroblasts ($124,767 \pm 14,285$ mRNA copies/ μ g RNA in normal versus $60,610 \pm 9,614$ copies/ μ g RNA in adhesion fibroblasts, respectively; $p < 0.003$). Both prolactin and androgen receptors expression was significantly higher in adhesion as compared to normal fibroblasts ($141,811 \pm 78$ PRL-R mRNA copies/ μ gRNA in adhesion versus $14,017 \pm 1,951$ copies/ μ g RNA in normal fibroblasts; $p < 0.001$; and $44,527 \pm 3,081$ A-R mRNA copies/ μ gRNA in adhesion versus $32,841 \pm 3,589$ copies/ μ g RNA in normal fibroblasts

Table 1 Sequences of the human hormone receptors primers studied

Locus	Sense (5'-3')	Antisense (3'-5')	Length
β -actin	AAG CAG GAG TAT GAC GAG TCC G	GCC TTC ATA CAT CTC AAG TTG G	559
Estradiol Rec.- α	TGC TGC TGG CTA CAT CAT C	CAG GAC TCG GTG GAT ATG G	158
Estradiol Rec.- β	ACA CTG GAG AAG GAA TAA G	GGA CTC AGT AAC TCA AGG	100
Progesterone Rec.	TCC AGC CAC ATT CAA CAC	CCG AAA CTT CAG GCA AGG	269
Prolactin Rec.	GTC AAT GCC ACT AAC CAG ATG	CAG GAG CGT GAA CCA ACC	195
Androgen Rec.	GCT GCT CCG CTG ACC TTA AAG AC	ACT GCT TCC TGC TGC TGT TGC	79

Fig. 1 **a** Study receptors in non-stimulated fibroblasts (*ER-α* estradiol-α receptor, *ER-β* estradiol-β receptor, *P-R* progesterone receptor, *PRL-R* prolactin receptor, *A-R* androgen receptor, * $p < 0.05$).

b Receptors expression at 10^{-10} M estradiol concentration. **c** Receptors expression at 10^{-8} M estradiol concentration. **d** Receptors expression at 10^{-6} M estradiol concentration

respectively; $p < 0.006$). Progesterone receptors were just slightly and non-significantly higher in adhesion fibroblasts ($28,045 \pm 737$ mRNA copies/ μ g RNA in adhesion versus $24,433 \pm 1,719$ copies/ μ g RNA in normal fibroblasts, respectively; ns).

In the fibroblasts cultured with 10^{-8} M estradiol concentration (which simulates the serum estradiol level reached during a human ovarian stimulation cycle) (Fig. 1c), estradiol-α and -β receptors expression was significantly higher in adhesion as compared to normal fibroblasts ($27,412 \pm 2,533$ ER-α mRNA copies/ μ g RNA in adhesion versus $2,464 \pm 429$ copies/ μ g RNA in normal fibroblasts; $p < 0.001$; and $183,192 \pm 5,289$ ER-β mRNA copies/ μ g RNA in adhesion versus $169,160 \pm 5,291$ copies/ μ g RNA in normal fibroblasts respectively; $p < 0.02$). Prolactin receptor expression became almost equal in the two cell types, as were progesterone receptors ($15,717 \pm 2,495$ PRL-R mRNA copies/ μ g RNA in normal versus $11,202 \pm 2,950$ copies/ μ g RNA in adhesion fibroblasts; ns; and $26,736 \pm 1,316$ P-R mRNA copies/ μ g RNA in normal versus $26,314 \pm 2,518$ copies/ μ g RNA in adhesion fibroblasts respectively; ns). Androgen receptor expression remained significantly higher in adhesion fibroblasts ($55,488 \pm 3,172$ mRNA copies/ μ g RNA in

adhesion versus $13,427 \pm 2,236$ copies/ μ g RNA in normal fibroblasts, respectively; $p < 0.001$).

Finally, in the fibroblasts cultured with 10^{-6} M estradiol concentration (which simulates the serum estradiol levels seen in the ovarian follicular fluid during ovarian stimulation with gonadotropins, and as such, will never be reached in the blood serum) (Fig. 1d), estradiol-α receptor expression became equal in the two cell types ($2,678 \pm 346$ mRNA copies/ μ g RNA in normal versus $3,309 \pm 782$ copies/ μ g RNA in adhesion fibroblasts, respectively; ns), estradiol-β receptors expression was significantly higher in adhesion as compared to normal fibroblasts ($106,142 \pm 1,898$ mRNA copies/ μ g RNA in adhesion versus $40,421 \pm 314$ copies/ μ g RNA in normal fibroblasts, respectively; $p < 0.001$), whereas prolactin, progesterone, and androgen receptors expression departed the previous trend and became higher in normal fibroblasts as opposed to adhesion fibroblasts ($54,196 \pm 848$ PRL-R mRNA copies/ μ g RNA in normal versus $25,473 \pm 2,781$ copies/ μ g RNA in adhesion fibroblasts; $p < 0.001$; $159,712 \pm 4,906$ P-R mRNA copies/ μ g RNA in normal versus $14,388 \pm 973$ copies/ μ g RNA in adhesion fibroblasts; $p < 0.001$; and $35,607 \pm 1,360$ A-R mRNA copies/ μ g RNA in normal versus $23,044 \pm 1,208$ copies/ μ g RNA in adhesion fibroblasts, respectively; $p < 0.001$).

Discussion

Using fibroblasts isolated from normal peritoneum and adhesion tissue obtained from three different patients, we demonstrated a differential expression of Estradiol- α , Estradiol- β , progesterone, prolactin, and androgen receptors in the absence or presence of increasing estradiol concentrations.

In particular, adhesion fibroblasts showed a significantly higher prolactin and androgen receptor expression with exposure to the physiologic early follicular estradiol concentration. Androgen receptor expression was still significantly higher in adhesion than in normal fibroblasts at the 10^{-8} M concentration, which mimics the peak serum estradiol concentration in an ovarian stimulation cycle. In this setting, also Estradiol- α receptor was elevated in adhesion fibroblasts, whereas prolactin receptor was not different than in normal fibroblasts. These responses could be interpreted as the normal priming effect of estradiol in the female genital tract tissues, but it was not seen in the normal peritoneal fibroblasts exposed to the same estradiol concentration. Moreover, this phenomenon was not seen for Estradiol- β and progesterone expression, which were no different in the two cell cultures. With exposure to the extremely high estradiol concentration reached in the follicular fluid during ovarian stimulation, expression of all receptors was lower in adhesion tissue than in normal fibroblasts except for Estradiol- β expression. Under the last condition, in normal fibroblasts, the up-regulatory effect of estradiol became noticeable on progesterone, prolactin, and androgen receptor expression; at the same time, estrogen receptors were down-regulated. In physiologic early follicular concentrations of estradiol, instead, normal fibroblasts showed an up-regulation of Estradiol- β receptors, as if they were intensifying certain effects of estradiol itself.

Previous studies suggested a protective role of progestins against adhesion development, possibly due to their immunosuppressive potential combined with the creation of a hypoestrogenic environment [6, 7]. Our results suggest that adhesion tissue fibroblasts would not be susceptible to progesterone, neither at low or high estradiol concentrations. The reported benefit of progesterone therapy versus adhesion formation could then be explained with the indirect effect of a hypoestrogenic environment on the activated fibroblasts. In our study, we quantified the total amount of progesterone receptors, and did not distinguish between the two receptor types, A and B. This could be a limitation in the interpretation of our results, because we could not distinguish between the inhibitory effects (mediated by progesterone receptor A) versus the stimulant effects (mediated by progesterone receptor B) of progesterone.

Except for a study from Frazier–Jensen which advocated a protective role of estrogen in adhesiogenesis, most animal studies have found in estrogens the main culprit in adhesion formation after abdominal surgery [8–12]. A recent study by Freeman et al., however, excluded either a preventive or a detrimental role of this hormone [13]. Our results seem to substantiate the no-role of estradiol ‘per se’ in adhesion formation in physiologic conditions, while finding in androgens, after estradiol cell-priming, a more suitable culprit. This new observation would also explain the partially protective effect of depot gonadotropin-releasing hormone-agonists in the formation of adhesions after peritoneal injury [14–17]. Indeed, these preparations act on the hypothalamus–pituitary gland–ovary axis, thus on the ovarian estrogenic/androgenic production.

Conclusions

Our study showed that, at physiologic or supraphysiologic estradiol concentrations (i.e. ovulation induction cycles), activated fibroblasts (adhesion tissue) seem to become more ‘receptive’ to prolactin, androgens, and estrogens than normal peritoneum fibroblasts. This susceptibility to prolactin and androgens seems to be more important in the early follicular phase conditions and in response to ovarian stimulation estradiol levels (10^{-10} M) (not prolactin), to decline at the highest estradiol concentrations. The susceptibility to estrogen through estradiol- α is highest at 10^{-10} M estradiol concentration. Prolactin has extensively been shown to mediate the inflammatory response throughout the body, and inhibit the immunologic reactions [18]. Our study would indirectly confirm a prolactin effect on adhesion formation with the finding of increased prolactin receptor expression in ‘activated’ fibroblasts in physiologic estradiol concentration. However, this effect gradually becomes attenuated in higher estradiol concentration environments. The physiologic anabolic effect of androgens and estrogen on the already activated fibroblasts would most probably enhance adhesion formation/consolidation after a peritoneal insult. Such association between adhesion formation in humans and androgens in vivo has never been reported in the literature.

In conclusion, our results may help in understanding the biologic effects of high serum estradiol on tissues. In particular, because of the anabolic effect of estradiol and androgens, the increased expression of estradiol- α and androgen receptors in adhesion fibroblasts may accelerate scar tissue formation in women during a healing process from inflammation, infection, and/or endometriosis.

References

1. Wiczak HP, Grow DR, Adams LA, O'Shea DL, Reece MT. Pelvic adhesions contain sex steroid receptors and produce angiogenesis growth factors. *Fertil Steril* 1998;69:511–6.
2. Diamond MP, DeCherney AH. Pathogenesis of adhesion formation/reformation: application to reproductive pelvic surgery. *Microsurgery* 1987;8:103–7.
3. Saed GM, Zhang W, Diamond MP. Molecular characterization of fibroblasts isolated from human peritoneum and adhesions. *Fertil Steril* 2001;75:763–8.
4. Henson MC, Shi W, Greene SJ, Reggio BC. Effects of pregnant human, non-pregnant human, and fetal bovine sera on human chorionic gonadotropin, estradiol, and progesterone release by cultured human trophoblast cells. *Endocrinology* 1996;137:2067–74.
5. Saed GM, Zhang W, Chegini N, Holmdahl L, Diamond MP. Alteration of type I and III collagen expression in human peritoneal mesothelial cells in response to hypoxia and transforming growth factor-beta1. *Wound Repair Regen* 1999;7:504–10.
6. Maurer JH, Bonaventura LM. The effect of aqueous progesterone on operative adhesion formation. *Fertil Steril* 1983;39:485–9.
7. Montanino-Oliva M, Metzger DA, Luciano AA. Use of medroxyprogesterone acetate in the prevention of postoperative adhesions. *Fertil Steril* 1996;65:650–4.
8. Frazier-Jessen MR, Mott FJ, Witte PL, Kovacs EJ. Estrogen suppression of connective tissue deposition in a murine model of peritoneal adhesion formation. *J Immunol* 1996;156:3036–42.
9. Wright JA, Sharpe-Timms KL. Gonadotropin-releasing hormone agonist therapy reduces postoperative adhesion formation and reformation after adhesiolysis in rat models for adhesion formation and endometriosis. *Fertil Steril* 1995;63:1094–100.
10. Grow DR, Coddington CC, Hsiu JG, Mikich Y, Hodgen GD. Role of hypoestrogenism or sex steroid antagonism in adhesion formation after myometrial surgery in primates. *Fertil Steril* 1996;66:140–7.
11. Sharpe-Timms KL, Zimmer RL, Joliff WJ, Wright JA, Nothnick WB, Curry TE. Gonadotropin-releasing hormone agonist (GnRH-a) therapy alters activity of plasminogen activators, matrix metalloproteinases, and their inhibitors in rat models for adhesion formation and endometriosis: potential GnRH-a-regulated mechanisms reducing adhesion formation. *Fertil Steril* 1998;69:916–23.
12. Ergeneli MH, Kusu E, Zeyneloglu HB, Haberal A, Erdogan M. GnRHa for adhesion formation after uterine serosal injury in an euestrogenic milieu. *J Reprod Med* 2000;45:35–8.
13. Freeman ML, Saed GM, Diamond MP. Hormone-independent ovarian influence on adhesion development. *Fertil Steril* 2002;78:340–6.
14. Henzl MR, Corson SL, Moghissi K, Buttram VC, Berqvist C, Jacobson J. Administration of nasal nafarelin as compared with oral danazol for endometriosis. A multicenter double-blind comparative clinical trial. *N Engl J Med* 1988;318:485–9.
15. Kuhlmann M, Gartner A, Schindler EM, Regidor PA, Buhler K, Schindler AE. Uterine leiomyomata and sterility: therapy with gonadotropin-releasing hormone agonists and leiomyomectomy. *Gynecol Endocrinol* 1997;11:169–74.
16. Canbaz MA, Ustun C, Kocak I, Yanik FF. The comparison of gonadotropin-releasing hormone agonist therapy and intraperitoneal Ringer's lactate solution in prevention of postoperative adhesion formation in rat models. *Eur J Obstet Gynecol Reprod Biol* 1999;82:219–22.
17. Roberts JE, Watkin KL, Tulandi T. Effects of leuprolide acetate on adhesion formation and wound healing in the rat model. *J Am Assoc Gynecol Laparosc* 2001;8:124–8.
18. Ben-Jonathan N, Mershon JL, Allen DL, Steinmetz RW. Extrapituitary prolactin: distribution, regulation, functions, and clinical aspects. *Endocr Rev* 1996;17:639–69.