Effects of the Selective Estrogen Receptor Modulator LY117018 on Growth Hormone Secretion: In Vitro Studies

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Sex steroids play an important role in modulating pulsatile growth hormone (GH) release, acting at both hypothalamic and pituitary level in both humans and experimental animals. Selective estrogen receptor modulators (SERMs) act as either estrogen receptor agonists or antagonists in a tissue-selective manner. In postmenopausal women, serum GH levels correlate positively with endogenous estradiol levels and insulin-like grwoth factor-I (IGF-I) is positively related to bone mineral density (BMD) at the spine and hip. The aim of the present study was to evaluate, for the first time, the direct effect of LY117018, an analog of raloxifene, on GH secretion from both human and rodent pituitary cells in vitro. Our results demonstrated that pharmacological concentrations of the raloxifene analog LY117018 can stimulate GH secretion through a direct action on the pituitary. LY117018 also showed an estrogen-like activity, inducing the proliferation of rat pituitary GH-secreting adenomatous cells (GH1).

SEX STEROIDS play an important role in modulating pulsatile growth hormone (GH) release, acting at both hypothalamic and pituitary level in both humans and experimental animals.[1,2] The physiological effects of estrogens on the pituitary, including regulation of hormone synthesis and secretion, are mediated by 2 separate, but related, forms of the nuclear estrogen receptor (ERα and ERβ), with distinct cell patterns of expression. The rat pituitary expresses also truncated estrogen receptor products (TERPs) that have not been detected in other tissues. ERβ is the major isoform expressed in human pituitary GH-secreting adenoma cells and may account for some of the actions of estrogens on GH previously thought to be indirect.[3,4] Estrogen-responsive elements have been located in the promoters of human growth-hormone releasing hormone (GHRH) receptor gene and human growth hormone secretagogue receptor gene.[5,6] Moreover, sex steroids modulate the expression of pituitary transcription factor-1 (Pit-1), which is specific to the anterior pituitary and involved in the expression and regulation of the GH as well as the prolactin gene.[7]

In humans, the stimulatory effects of estrogens on GH secretion have been well documented. In rodents, whereas androgens consistently facilitate the pituitary GH response to GHRH, contradictory data have been obtained about the effects of estrogens[8]: in fact, Simard et al reported that estradiol exposure stimulates in vitro basal and GHRH-stimulated GH secretion by pituitaries obtained from female rats,[9] whereas Fukata and Martin indicated no influence of sex steroids on GHRH-induced GH release under the same conditions.[10] In vivo, it has been reported that estradiol treatment increases baseline GH levels in the male rat, without influencing the pulse height,[11] but estradiol treatment of castrated male rats leads to reduced in vitro GH secretion, similar to that reported in female animals.[12] In humans estrogens have been shown to enhance the GH response to several stimuli.[13]

Selective estrogen receptor modulators (SERMs) act as either estrogen receptor agonists or antagonists in a tissue-selective manner. Compounds of this class act as agonists in bone with inhibition of bone resorption, and in the cardiovascular system with a sustained reduction of markers of cardiovascular risk as low-density lipoprotein (LDL)-cholesterol and plasma homocysteine in postmenopausal women. Moreover, they act as estrogen antagonists in the breast, reducing the risk of breast cancer.[14,15]

The effect of tamoxifen on GH secretion has been studied both in vitro and in vivo, in humans and experimental animals. Tamoxifen has been shown to inhibit GH release from immature lamb pituitary cultures[16] and to attenuate pulsatile growth hormone secretion in rats[17]; conversely, it has been reported to increase serum GH levels, while decreasing insulin-like growth factor-I (IGF-I) levels, when chronically administered to acromegalic patients.[18] In postmenopausal women with breast cancer, chronic tamoxifen administration significantly decreases serum IGF-I levels, with inhibited[19] or unaffected[20] GH response to GHRH. The nonsteroidal benzothiophene derivative, raloxifene, is the first SERM that has been approved for the prevention and treatment of postmenopausal osteoporosis and appears to have different pharmacological properties as compared to tamoxifen. Unlike tamoxifen, it lacks uterotrophic activity. In a recent clinical trial, raloxifene reduced the risk of invasive breast cancer in postmenopausal women with osteoporosis without increasing the risk of endometrial cancer.[14,21,22] In addition to direct effects on bone cells, raloxifene may act as an estrogen agonist via changes in hormonal homeostasis. In postmenopausal women, serum GH levels correlate positively with endogenous estradiol levels and IGF-I is positively related to bone mineral density (BMD) at the spine and hip.[1,23,24] No data are available on the effects of raloxifene at the pituitary level on GH secretion. Recently, it was shown that raloxifene administration is associated with decreased serum IGF-I levels in postmenopausal women with osteoporosis both before and

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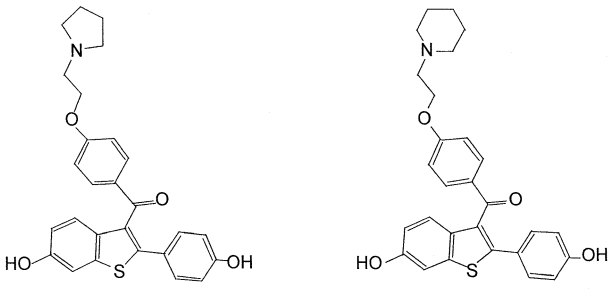


Fig 1. The structures of raloxifene and LY117018.

after an injection of recombinant human GH.[25] Actually, this association may be not accompanied by any inhibitory effect on GH release from pituitary and could be the result of a decreased hepatic response to GH.

The aim of the present study was to address the role of SERMs on somatotroph function focusing on the activity of LY117018, an analog of raloxifene, on GH secretion from both human and rodent pituitary cells in vitro. Since GH and IGF-I are both critical for the maintenance of skeletal mass,[26] GH inhibition may be a drawback in the antiosteoporotic use of those SERMs which are pure estrogen antagonists at the pituitary level.

MATERIALS AND METHODS

Drugs

LY117018 (Fig 1) was kindly provided by Eli Lilly Co (Indianapolis, IN). Tamoxifen and 17β-estradiol were purchased by Sigma (St Louis, MO). GHRH (1-29) was purchased from Serono (Milan, Italy).

The doses of SERMs tested in vitro are representative of steady-state levels of tamoxifen, our reference substance, in breast cancer patients under chronic treatment, according to Maalab et al.[16] Drugs, except for GHRH, were dissolved in ethanol. The final concentration of vehicle in the culture medium never exceeded 0.01%. The same amount of vehicle was added to the control wells.

GH1 Cell Culture

The GH-secreting rat pituitary adenoma cells were plated at 50,000 cells per well in 24-well tissue culture plates (Costar, Cambridge, MA) in 1 mL culture medium (Ham's F10 supplemented with 10% fetal calf serum, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin) and incubated for 48 hours in a humified atmosphere of 95% air, 5% CO_2, at 37°C.

Effect of LY117018 and Tamoxifen on GH Secretion

The growth medium was removed by aspiration and cells were washed 3 times with phosphate-buffered saline (PBS). Medium was changed to Ham's F10 without serum and cells were treated for 6 or 24 hours with test substances. At the end of the incubation the medium was collected and stored frozen (−20°C) for later GH measurements.

Effect of LY117018 and 17-β Estradiol on Cell Proliferation

Cells were plated at 120,000 cells per well in 24-well plates. Twenty-four hours after plating, the medium was changed to Ham's F10 supplemented with 2% fetal calf serum (FCS) and cells were treated with test substances. Proliferation was measured by methyl [^{3}H]thymidine pulse labeling (specific activity, 185 GBq/mmol; Amersham, Milan, Italy; 0.5 μCi/well for 12 hours) 3 days after their administration. Cells were detached from the plates using trypsin-EDTA, then harvested by means of a cell harvester for 96-well plates and collected on a filter paper. [^{3}H]thymidine incorporation was finally measured by liquid scintillation counting (Wallac, Turku, Finland).

Primary Rat Pituitary Cell Culture

Adult male Sprague-Dawley rats (300 to 350 g) were decapitated and the pituitaries were removed under sterile conditions. Tissues were washed in PBS, then minced and enzymatically dissociated using 0.35% collagenase and 0.25% trypsin (both from Sigma) for 30 minutes at room temperature. Cell suspension was centrifuged and the pellet resuspended in high-glucose Dulbecco's modified Eagle Medium (D-MEM) supplemented with 10% FCS, 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μg/ mL streptomycin. For primary cultures, 50,000 cells per well were seeded in 24-well culture plates (Costar) in 1 mL medium and incubated for 96 hours in a humified atmosphere of 95% air/5% CO_2, at 37°C.

Effect of LY117018 and Tamoxifen on GH Secretion

The growth medium was removed by aspiration and cells were washed 3 times with PBS. Incubations were carried out through 6 or 24 hours, in serum-free D-MEM supplemented with LY117018 or tamoxifen (from 0.01 to 1 μmol/L) with or without GHRH (1-29) 10 nmol/L (Geref, Serono, Milan, Italy). Coincubations experiments with 17-β estradiol at different doses (from 0.01 to 1 μmol/L) and LY117018 0.1 μmol/L were carried out for 6 hours in D-MEM without serum. At the end of each incubation, the medium was collected and assayed for GH content.

Human GH-Secreting Pituitary Adenomas

Patients. Adenomatous tissue obtained after transphenoidal surgery from 14 acromegalic patients was studied. The clinical characteristics of patients are presented in Table 1. All of the adenomas were classified as pure GH-secreting tumors based on the results of immunohistochemistry.

Adenoma cell cultures. Primary cell cultures were established as previously reported. Briefly, adenomatous tissues were washed with PBS and dissected into small pieces. The tissue fragments were mechanically and enzymatically dispersed using 0.25% trypsin and 0.02% EDTA at pH 7.3. Dispersed cells were centrifuged and resuspended in sterile culture medium. The cells were diluted with cultured medium and plated in 24-well plates (Costar) at an initial concentration of 50,000 per well. The initial growth medium was (D-MEM) supplemented with 10% FCS, 2 mmol/L glutamine, and antibiotics. The cells were grown at 37°C in an atmosphere of 5% CO_2. After 3 days, the cells were washed 3 times with PBS and fresh growth medium was added.

Effects of LY117018 and Tamoxifen on GH Secretion

LY117018 and tamoxifen were both tested at the doses 0.01, 0.1, and 1 μmol/L. Five days after plating, when usually an 80% confluent monolayer was obtained, cells were washed 3 times with PBS and 6-hour incubations or time-course experiments (24 to 72 hours) were carried out in D-MEM without serum. Coincubations experiments with 17-β estradiol at the maximal effective dose (1 μmol/L) and LY117018 at different doses (from 0.01 to 10 μmol/L) were performed through 6 or 72 hours in the same medium. At the end of each incubation, medium was collected and assayed for human GH content.

Table 1. Clinical Characteristics of Acromegalic Patients and In Vitro GH Secretion by Adenomatous Dispersed Cell

Adenoma No.	Age (yr)	Sex	Tumor Size	GH (ng/mL)	IGF-I (ng/mL)	GH Secretion (ng/mL)
1	54	F	Macro-	16.2	>1,000	910
2	54	F	Macro-	NA	NA	600
3	36	M	Micro-	13.5	NA	626
4	64	M	Macro-	21.7	NA	1,118
5	45	F	Macro-	2.1	NA	170
6	47	M	Micro-	10.5	530	840
7	39	M	Micro-	7.5	660	1,667*
8	60	M	Micro-	21.2	629	5,966*
9	38	F	Macro-	18.9	545	1,701*
10	59	F	Macro-	2.8	545	373
11	40	M	Macro-	14.8	547	164
12	59	F	Micro-	3.7	712	150
13	59	F	Micro-	8	462	1,500
14	70	M	Macro-	14.3	>760	820

NOTE. Presurgical plasma GH, IGF-I levels, and mean basal GH concentration in the medium after 6 or 24 hours *incubation are reported.

Abbreviation: NA, not available.

GH Determination

Rat GH concentrations in cell culture media were determined by radioimmunoassay (RIA) with reagents kindly provided by the National Institute of Diabetes and Digestive and Kidney Disease (Bethesda, MD; minimum detectable dose, 0.4 ng/mL). Commercial kits were used for the estimation of human GH concentration in collected media (hGH RIA kit; minimum detectable dose, 0.5 ng/mL; ICN Biomedicals, Asse-Relegem, Belgium). Inter- and intra-assay variability were lower than 5%.

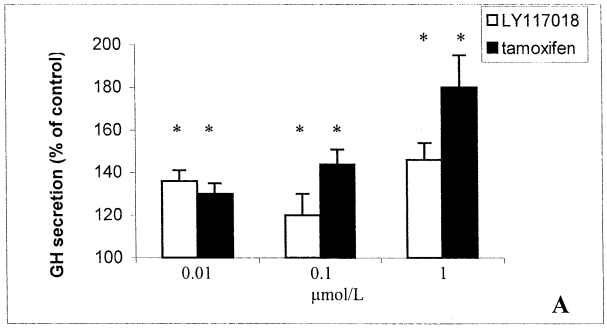
Statistics

Unless otherwise stated, data are reported as the mean ± SEM. For each experiment, the statistical significance of the differences between the mean basal GH secretion and GH secretion in the presence of different stimuli was assessed by *t* test preceded by 1-way analysis of variance (ANOVA) where appropriate, with statistical significance defined as $P < .05$. Then, data were reported as a percentage of the mean basal GH concentration in each experiment, because of the variability of the GH secretion in the different cell cultures.

RESULTS

Effects of LY117018 or Tamoxifen on GH Secretion by Dispersed Pituitary Cells

Male rat pituitary primary cell cultures. During 6-hour as well as 24-hour exposure of the cells to increasing concentrations of SERMs (0.01 to 1 μmol/L) there was an increase of basal GH secretion. The stimulatory effect on GH, expressed as a percent of the respective control, was slightly reduced after 24 hours as compared to a short time of incubation (6-hour GH peak, LY117018 1 μmol/L: 146% ± 10%; tamoxifen 1 μmol/L: 175% ± 15%; $P < .01$; 24-hour GH peak, LY117018 1 μmol/L: 118% ± 12%; tamoxifen 1 μmol/L: 120% ± 10%; $P < .05$) and the effect of tamoxifen no longer appeared dose-dependent (Fig 2). LY117018 and tamoxifen were also able to stimulate significantly ($P < .05$) the GH response to 10 nmol/L GHRH during both incubation periods (6-hour GH peak, GHRH 10 nmol/L: 460% ± 7%; GHRH 10 nmol/L + LY117018 1 μmol/L: 700% ± 10%; GHRH 10 nmol/L +



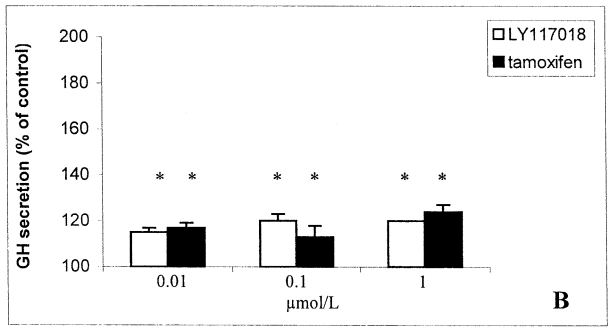
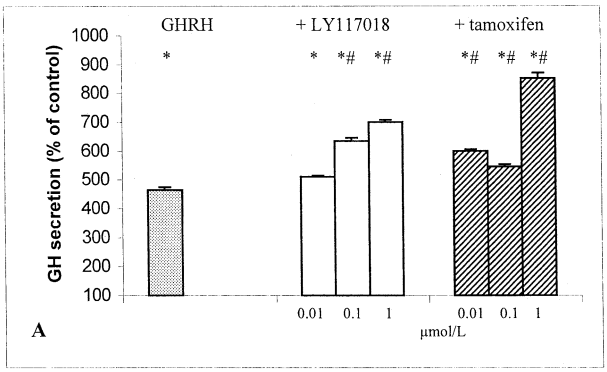


Fig 2. GH secretion in male adult rat primary pituitary cultures. Effects of LY117018 and tamoxifen on basal somatotroph activity. (A) Six-hour incubation; (B) 24-hour incubation. Results are expressed as % hormone secretion *v* vehicle-treated control wells (mean GH concentration in 4 to 5 wells ± SE; **P* < .05 *v* control wells; mean basal GH concentration: over 6 hours, 250 ng/mL; over 24 hours, 980 ng/mL).



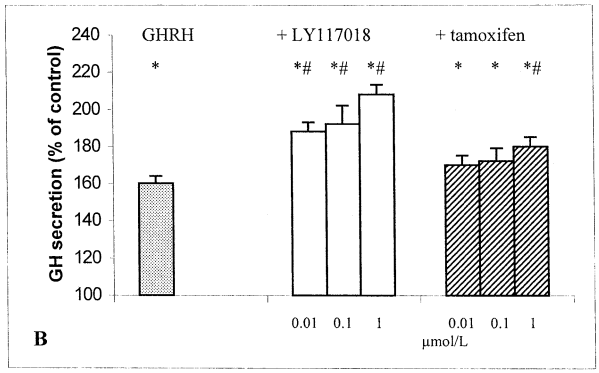


Fig 3. Effects of LY117018 and tamoxifen on 10 nmol/L GHRH-induced GH secretion in male adult rat primary pituitary cultures. (A) Six-hour incubation; (B) 24-hour incubation. Results are expressed as % hormone secretion vs vehicle treated control wells (mean GH concentration in 4 to 5 wells ± SE; *$P < .05$ *v* vehicle-treated control wells; #$P < .05$ *v* GHRH-treated wells).

tamoxifen 1 μmol/L: 900% ± 20%) (Fig 3); at 24 hours, the enhancement of GHRH-induced GH release was attenuated and, for tamoxifen, the only significant effect was at the highest concentration tested.

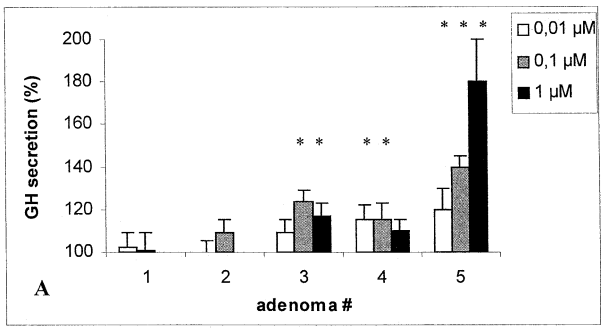
Rat GH-secreting adenoma cell line (GH1). The stimulatory effect of LY117018 (0.01 to 1 μmol/L) on GH secretion was not dose-related; at 6 and 24 hours, by normalizing the data as a percent of the respective control, the GH peak was negligible as compared to that observed in the rat pituitary primary cultures (6-hour GH peak, LY117018 0.1 μmol/L: 115 ± 3%; $P < .05$). Moreover, tamoxifen did not affect significantly GH secretion, in contrast with the stimulatory effect in the previous model system. Over 72 hours, the GH-releasing effect of LY117018 was more clear-cut than over short-term incubation, whereas tamoxifen was still ineffective (GH peak, LY117018 1 μmol/L: 160% ± 8%, $P < .05$; tamoxifen 1 μmol/L: 107% ± 5%).

Human GH-secreting pituitary adenomas. During acute (6 hours) exposure to increasing concentrations of SERMs (0.1 to 1 μmol/L), LY117018 stimulated significantly GH secretion in 3 adenoma cell cultures (no. 3, 4, and 5) out of 5 (LY117018 0.1 μmol/L: 139% ± 24% as mean GH ± SEM) (Fig 4A). Tamoxifen stimulated GH release by 3 adenomas (no. 3, 4, and 6) out of 4 and the maximal effective dose was 1 μmol/L (mean GH peak, 120% ± 3% as mean value ± SEM) (Fig 4B). We compared directly the effects of LY117018 and tamoxifen in 3 different adenoma cell cultures and we observed a completely concordant GH response, with 2 "responders" (no. 3 and 4) and 1 "nonresponder" (no. 2) adenoma.

We have investigated the effects of long-term exposure (24 and 72 hours) as compared to short-term incubation with SERMs in 3 different adenoma cell cultures (no. 7, 8, and 9). The effects of LY117018 and tamoxifen were still concordant (Fig 5A and B, respectively): they both stimulated GH release by 2 adenomas (no. 7 and 8) out of 3 during the 24-hour incubation period. In one case, the GH-releasing effect lasted up to 72 hours (no. 8).

Effects of LY117018 on Estrogen-Stimulated GH Secretion From Pituitary Dispersed Cells

Rat pituitary primary cells. 17-β Estradiol significantly enhanced the accumulation of GH in the medium after 6 hours of treatment (GH peak, 1 μmol/L: 140% ± 12% *v* vehicle, $P < .05$). Interestingly, in coincubation experiments with 17-β estradiol and LY117018 0.1 μmol/L we did not observe any stimulatory effect of 17β estradiol (Fig 6).



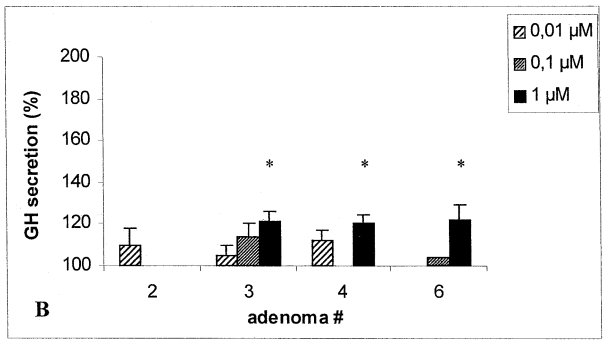


Fig 4. Effects of (A) LY117018 or (B) tamoxifen on spontaneous GH release by human GH-secreting pituitary adenoma cells (adenoma # refers to the numbers shown in Table 1): dose-response curve. Incubations were carried out through 6 hours in serum-free D-MEM. Results are expressed as % hormone secretion *v* vehicle-treated control wells (mean GH concentration in 3 wells ± SE; *$P < .05$ *v* control wells).

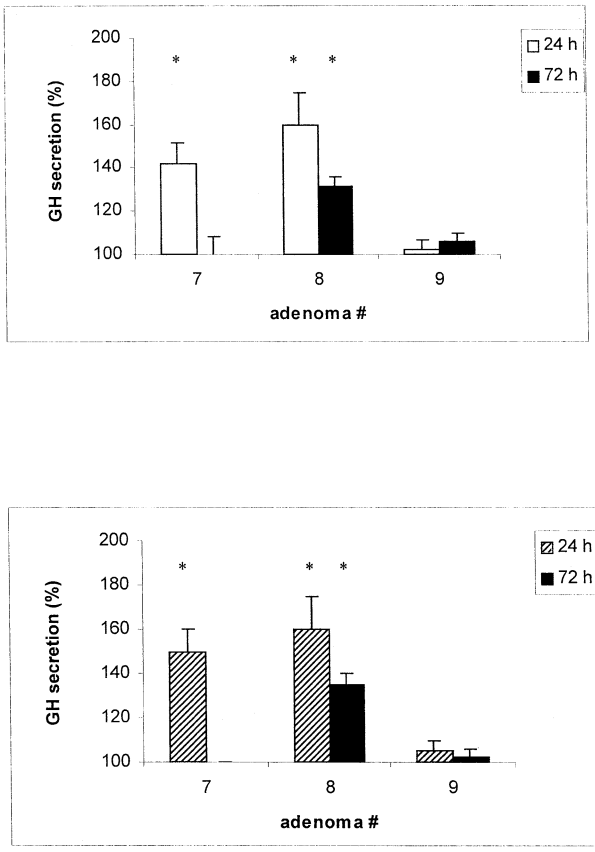


Fig 5. Effects of (A) 0.1 μmol/L LY117018 or (B) 0.1 μmol/L tamoxifen on GH secretion by human GH-secreting pituitary adenoma cells (adenoma # refers to the nymbers shown in Table 1): time-course experiments. Medium was collected at 24 or 72 hours after the drug administration. Results are expressed as % hormone secretion *v* vehicle-treated control wells (mean GH concentration in 3 wells ± SE; **P* < .05 *v* control wells).

Human GH-secreting pituitary adenoma cells. The experiments were performed in 5 different adenoma cell primary cultures (no. 10 through 14). 17-β Estradiol clearly induced the accumulation of GH in the medium at the concentration 1 μmol/L (mean GH peak ± SEM in 5 different tumor cell cultures: 6 hours, 140% ± 10%; 72 hours, 130% ± 20% *v* medium alone), whereas it was ineffective at lower concentrations (estradiol 0.1 μmol/L, 6 hours: 104% ± 15%; 72 hours: 94% ± 7% as mean GH values in 3 different tumor cell cultures). When concomitantly added to the growth medium, LY117018 was able to antagonize the stimulation caused by estradiol. Actually, 0.1 μmol/L LY117018, but not 0.01 μmol/L, inhibited the GH stimulatory effect of 1 μmol/L estradiol (Fig 7).

Effects of LY117018 and 17-β Estradiol on GH1 Cell Proliferation

17-β Estradiol increased dose-dependently GH1 cell proliferation. LY117018 also stimulated [^{3}H]thymidine incorporation by GH1 cells, with maximal stimulation about 2-fold greater than basal incorporation (Fig 8).

DISCUSSION

The direct effects of tamoxifen, the first SERM that has proven useful for the blockade of estrogen receptors in breast tumor cells, at the pituitary level are controversial. Tamoxifen has been reported to stimulate prolactin production in primary cell cultures of male rat pituitary gland acting as partial estrogen agonist.[28] In contrast, tamoxifen suppressed growth and secretion of prolactin-secreting pituitary tumors in rats, and 17-β estradiol-induced cell proliferation of human pituitary adenomas in vitro.[27] Direct effects of tamoxifen on GH secretion by pituitary cells in vitro have been also reported: tamoxifen inhibited GH secretion from immature lamb pituitary cultures during acute as well as chronic treatment, and such inhibitory effect was assumed to be consistent with the lower serum GH levels in breast cancer patients receiving tamoxifen than those receiving placebo.[16] However, the same drug increased or left unchanged serum GH levels when chronically administered in acromegalic patients and stimulated GH secretion from human pituitary GH-secreting adenomas in vitro.[18,30,31]

Raloxifene is known to have some different pharmacological properties as compared to tamoxifen and no data are available on its direct effects on somatotrophs. Here we demonstrated, for the first time, that LY117018 stimulates GH secretion in 2 different in vitro models, acting as an estrogen agonist at this level. The interpretation of the data from human adenomas and rat normal or adenomatous cells is important to establish the physiological significance of the observed in vitro effects due to the possibly paradoxical behavior of adenoma cells.[32,33]

In male rat pituitary dispersed cells, both LY117018 and tamoxifen increased spontaneous GH release and enhanced the maximal GH response to GHRH after a 6-hour as well as 24-hour incubation period. The stimulatory effects of the SERMs on basal and GHRH-induced GH secretion, if calculated as a percent of the respective control, were not significantly different; they are likely to play a permissive role on GH secretion, acting independently from the GHRH signaling pathway. Our data differ from those of Malaab et al,[16] who found

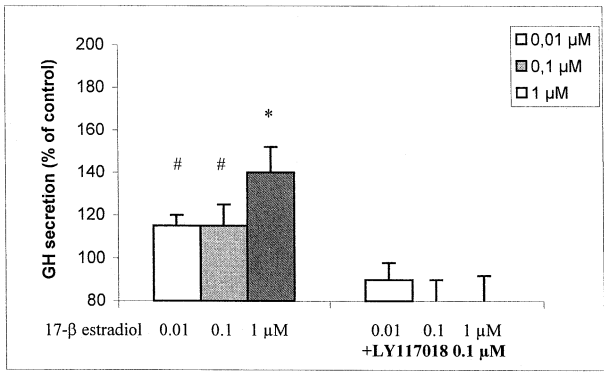


Fig 6. GH response to 17-β estradiol in male adult rat pituitary primary cell cultures: dose-response curves in medium without serum (vehicle) and in presence of 0.1 μmol/L LY117018 (vehicle + LY117018). Incubation was carried out through 6 hours. Results are expressed as % hormone secretion *v* vehicle-treated control wells (mean GH in 3 wells ± SE; **P* < .05; #*P* < .1 *v* control wells).

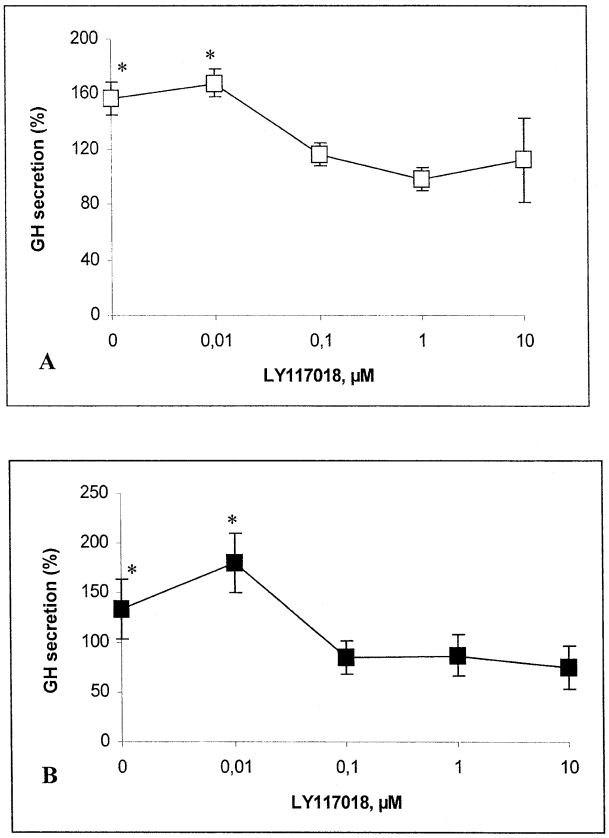


Fig 7. Mean GH response to 1 μmol/L 17 β-estradiol in human pituitary GH-secreting adenoma cell cultures in medium without serum and in presence of different doses of LY117018. Data are expressed as % hormone secretion vs vehicle treated control wells and each point represents the mean ± SEM of results from 5 different adenomas (from #10 to #14). (A) Six-hour incubations; (B) 72-hour incubations (*$P < .05$; *v* control wells).

tamoxifen behaved as inhibitor of GH release in vitro at the same doses tested by us. Actually, the 2 model systems are quite different since they used immature lamb pituitary cultures and we used adult male rat pituitaries; moreover, they used growth medium supplemented with fetal bovine serum (FBS), whereas our incubations were performed in medium without serum.

LY117018, but not tamoxifen, produced a weak stimulatory effect on GH secretion by the GH-secreting rat pituitary adenoma cell line, GH1. GH1 cells showed reduced GH response to LY117018 as compared to rat pituitary primary cultures. Since pituitary cell lines have been derived from single cells by clonal selection, GH1 cells are presumably homogeneous population of tumoral somatotrophs, whereas pituitary primary cultures are a mixture of cells with phenotypic heterogeneity, ie, mammosomatotrophs, somatotrophs, or nonsecreting cells; this difference between the 2 model systems could be related to the different response to SERMs.

Human GH-secreting pituitary adenoma cells express the estrogen receptor genes. The nuclear estrogen receptor acts as a dimer to modulate gene transcription. It has been reported that at least 60% to 70% of human GH-secreting pituitary adenomas express estrogen receptor and, in this type of tumor, the ERβ isoform is more frequently expressed than ERα.[3] In this work, we have studied the direct effects of LY117018 and tamoxifen on GH secretion by a large number of cultured human GH-secreting pituitary tumors. Six of 9 adenomas were clearly responsive to both LY117018 and tamoxifen with a significant stimulatory effect on GH release. Short incubation periods were sufficient to increase significantly the growth medium GH content. Three adenomas were not responsive to either drug, but we did not observe any inhibitory effects of the SERMs on GH release. We did not observe any correlation between the responsiveness of the adenomas and the gender of the patients. Actually, experiments have been performed after a wash-out of 48 hours of the adenomatous cells in medium supplemented with FCS and this condition could have suppressed some gender-related differencies.

We have not investigated the effects of SERMs on GHRH-induced GH secretion by rat or human pituitary adenoma cells because, in these cells, the GHRH signaling pathway is frequently activated by *gsp* mutations or high levels of the phosphorylated cyclic adenosine monophosphate (cAMP)-regulated factor CREB, which results in constitutive GH hypersecretion.[33,34] Apparently, this feature does not prevent the human somatotrophs responding to the stimulatory effects of SERMs, but it may attenuate the SERM-stimulated GH release in GH1 cells as compared to normal rat somatotrophs.

In order to establish the relationship between the actions of estrogen and raloxifene at the pituitary level, we evaluated the in vitro effect of concomitant 17-β estradiol and LY117018 on GH secretion by normal rat somatotrophs and human GH-secreting pituitary adenoma cells. 17-β Estradiol caused a significant rise in GH release at a relatively high concentration, with the same time-course as LY117018. Interestingly, the coincubation of 17-β estradiol and LY117018 leads to a loss of the GH-releasing effect. It has been reported that, upon binding hormone, estrogen receptor undergoes a conformational change and the activated receptor can interact as a dimer with DNA on target genes.[14] Recent data indicate that different ligands, such as raloxifene and tamoxifen, determine different receptor conformations that are transcriptionally active or inactive in a

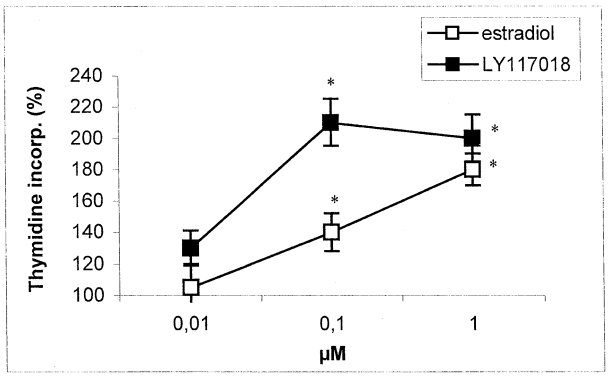


Fig 8. Effects of 17-β estradiol or LY117018 on GH1 cell proliferation: dose-response curves (0.01 to 1 μmol/L). *$P < .05$ *v* control wells.

tissue-selective manner.[35] As a possible explanation of our data, we postulate that in rat as well as human GH-secreting pituitary cells, the concomitant native ligand estradiol and LY117018, both at high concentrations and presumably saturating levels, may create different ligand-ER complexes that could not either dimerize or activate the transcriptional cascade up to GH secretion as well as that observed with either 17-β estradiol or LY117018 alone. Clearly, more pharmacological studies are needed to support this hypothesis.

In conclusion, our results demonstrated that a pharmacological concentration of the raloxifene analog LY117018 can stimulate GH secretion through a direct action on the pituitary. The interaction between LY117018 and 17-β estradiol, with no additive effects but the blockade of the stimulatory effect of estrogens by simultaneous incubation with LY117018, suggests a competition between these ligands for the binding domain of the estrogen receptor and supports the involvement of the estrogen receptor in the action of SERMs at the pituitary level. Moreover, the clear-cut proliferative effect of LY117018 in the comparative studies with 17-β estradiol on GH1 cells further support the estrogen-like activity of the raloxifene analog in pituitary cells.

GH stimulates bone growth and remodeling through interaction with specific GH-binding sites on osteoblasts and indirectly via an induction of endocrine or autocrine/paracrine IGF-I.[36] Besides a direct action on the skeleton, GH effects on bone and mineral metabolism may also involve intestinal calcium adsorption,[37] hydroxylation of 25-hydroxyvitamin D, and muscle strength.[38] Thus, the in vitro results reported in the current study suggest that the beneficial effects of raloxifene on bone may be, in part, sustained by the estrogen-agonist effects on GH release at the pituitary level.

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