Changes of Cell Morphology and Prolactin Secretion Induced by 2-Br-α-Ergocryptine, Estradiol, and Thyrotropin-releasing Hormone in Rat Anterior Pituitary Cells in Culture

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ABSTRACT The secretion of prolactin in cultured pituitary cells was studied in correlation with the cellular changes induced by stimulatory or inhibitory agents. The techniques used in this study were: radioimmunoassay, immunocytochemistry, scanning (SEM) as well as transmission (TEM) electron microscopy. Prolactin secretion was stimulated by 17β-estradiol (10 nM) as well as thyrotropin-releasing hormone (TRH) (3 nM) and inhibited by 2-Br-α-ergocryptine (CB-154) (1 μM). The total prolactin (release and cell content) increased between 2 and 8 d of estradiol treatment, indicating an increase of both synthesis and release of prolactin. This finding was in agreement with TEM observations because, in estradiol-treated prolactin cells, the Golgi saccules were distended and Golgi elements were increased, thus indicating increased synthetic activity of these cells. The addition of TRH over a 4-h period resulted in a significant degranulation of prolactin cells. In contrast, prolactin secretory granules became accumulated in the cells after CB-154 treatment for a period ranging from 4 to 24 h. In agreement, light microscope immunocytochemistry showed an increased reaction for prolactin after short-term (<24 h) incubation with CB-154. Because prolactin cells represent ~70% of the glandular cell population as revealed by immunocytochemistry, it was then possible to observe the changes of cell surface by SEM. In most cells, estradiol and TRH led to an increase in the number and prominence of microvilli and blebs, whereas CB-154 treatment resulted in a slightly decreased number of microvilli and an increased occurrence of membrane foldings. This report thus provides morphological evidence for the stimulatory effects of estradiol and TRH, and the inhibitory effects of CB-154 on prolactin secretion in pituitary cells in primary culture. These data, moreover, show that acute changes in secretory activity of prolactin-secreting cells are accompanied by marked changes of their morphological characteristics.

The secretion of prolactin in the anterior pituitary gland is under predominant inhibitory control by the hypothalamus (30). Recent data indicate that dopamine may be the main or even the only inhibitory substance of hypothalamic origin involved in the control of prolactin secretion (11, 29, 37). This role of dopamine is also supported by the finding that 2-bromo-α-ergocryptine (CB-154), a potent dopamine agonist, and other ergot derivatives are potent inhibitors of prolactin secretion in vivo as well as in vitro (16, 26, 31, 33).

The stimulatory influence of the hypothalamus can, at least partly, be mediated by thyrotropin-releasing hormone (TRH). This tripeptide is known to stimulate prolactin secretion in vivo (7, 14) and in vitro (15, 38). Prolactin secretion is also well known to be increased after estrogen treatment in man (10, 39).
and the rat (13). Recently, estrogens have been found not only to stimulate prolactin secretion but also to reverse the inhibitory effects of dopamine agonists (including CB-154) by a direct action at the pituitary level (26, 34).

Pituitary cells in primary culture (25, 38) offer an attractive system for the study of cells by scanning electron microscopy and permit a precise correlation with changes of pituitary hormone secretion induced by various stimulatory or inhibitory agents. In preliminary reports (1, 2), we have recently described a good correlation between the surface morphology of anterior pituitary cells in primary culture and prolactin and thyroid-stimulating hormone (TSH) secretion after short- and long-term treatment with estrogens, CB-154, triiodothyronine, TRH, and colchicine (3). In the present report, we have used immuno- 

andos and microchemical localization of prolactin, scanning and transmission electron microscopy, and radioimmunoassay data for each experimental condition, to describe the effects and interactions of 17ß-estradiol (E2), TRH, and CB-154 in this system at the cellular level.

MATERIALS AND METHODS

Materials

CB-154 was kindly provided by Sandoz Pharmaceuticals (East Hanover, N. J.) whereas TRH and E2 were purchased from Peninsula Laboratories Inc. (San Carlos, Calif.) and Steraloids, Inc. (Pawling, N. Y.), respectively. Media and sera for tissue cultures were obtained from Grand Island Biological Co. (Grand Island, N. Y.), and glutaraldehyde (special grade) was a product of Ladd Research Industries, Inc. (Burlington, Vt.).

Preparation of Dispersed Anterior Pituitary Cells

Adult female Sprague-Dawley rats weighing 200-400 g (obtained from Canadian Breeding Farms, St. Constant, Quebec) at random stages of the estrous cycle were used for the preparation of primary cultures of anterior pituitary cells as previously described (25, 38). The dissociated cells (5.5-9.0 x 10^6) were plated in Corning Glass Works, Science Products Div. (Corning, N. Y.), polystyrene Petri dishes (35 x 10 mm) in 1.5 ml of Dulbecco's modified Eagle's medium (DMEM) containing 2.5% fetal calf serum and 10% horse serum (dextran-coated charcoal adsorbed, 25), nonessential amino acids, 50 U/ml penicillin, and 50 µg/ml streptomycin. Cell counts were done (using a hemocytometer) during plating to ensure that all the Petri dishes in an experiment were seeded with the same number of cells. The cells were maintained at 37°C in a water-saturated atmosphere of 95% O₂ and 5% CO₂ for up to 12 d.

Determination of Cell Viability

For a cell culture to be representative of the whole population of a tissue, cell viability after dissociation must be high enough so that no major fraction of the cells is excluded. The percent of viable cells in the dispersed cell suspension was determined by the trypan blue method. 1% trypan blue (dissolved in HEPES buffer) was mixed with an equal amount of cell suspension. Viable cells were those that were not stained blue, and their percentage was calculated for at least four aliquots per culture. Cell viability was at least 75% in the cultures.

Incubation Procedure

In time-course experiments, the cells were treated at 24-h intervals with freshly prepared CB-154 (sterile stock solution of 1.5 x 10⁻⁴ M in 1% ethanol-0.9% NaCl added in a volume of 10 µl/Petri dish. Such a low concentration of ethanol did not affect the radioimmunoassay data. E2 stock solution (1.5 x 10⁻⁴ M in 0.09% NaCl-1% ethanol) was added at 48-h intervals in the same volume. While the cells were maintained in culture for 12 d, they were allowed to attach for 3 d and were then treated as indicated in the figure legends. After attachment of the cells to the Petri dishes, the incubation medium was removed and replaced with fresh medium (with or without additions) at 48-h intervals. In all experiments, after the preliminary incubation, the cells were washed four times with sterile serum-free DMEM, and the final incubations were performed in triplicate in the absence or presence of TRH for 4 h at 37°C. At the end of incubations, the media were centrifuged at 100 g for 7 min at 4°C and the supernatants were stored at -20°C until assayed. To determine the prolactin cell content, 1 ml of 50 nM Na₂CO₃ with 2 mM EDTA was added to the Petri dishes. Subsequently, the Petri dishes were rinsed with 1 ml of acidified DMEM, and the pooled suspensions were filtered, thawed, centrifuged at 100 g for 7 min at 4°C, and the supernate was stored at -20°C until assayed.

Hormone Assays

Prolactin was measured in duplicate by a double-antibody radioimmunoassay (15) using rat prolactin 1-2 and rabbit antisera (anti-rat prolactin-S-3) kindly supplied by Dr. A. F. Parlow for the National Institute of Arthritis and Metabolic Diseases, Rat Pituitary Hormone Program. Radioimmunoassay data were analyzed with a Hewlett-Packard calculator (model 9830, Hewlett-Packard Co., Palo Alto, Calif.) using a program based on model II of Rodbard and Lewald (35).

The statistical significance was determined according to the multiple-range test of Duncan-Kramer (24). All data are presented as the means ± SEM of duplicate measurements of triplicate dishes.

Immunocytochemistry

Petri dishes were rinsed four times with Tris-buffered saline (Tris, 0.05 M, NaCl 0.9%, pH 7.6) and fixed in situ with Bouin's fixative for 20 h at 4°C or with 4% buffered paraformaldehyde as previously described (4). Cells were subsequently processed for the immunocytochemical localization of prolactin by the unlabeled antibody method and the peroxidase-antiperoxidase complex technique (PAP) according to Sternberger (36) and as previously described (4). The dilution of the rabbit anti-prolactin serum (same lot as that used in the hormone assays) was 1/1,000-1/2,000. Diaminobenzidine (Sigma Chemical Co., St. Louis, Mo.) (22) was used as hydrogen donor for the localization of peroxidase. The specificity of the immunocytochemical reaction was tested by incubating the cells either with antisera previously absorbed with excess antigen or with sera from immunized rabbits. Finally, to eliminate the possibility that endogenous peroxidases might be participating in the reaction, cells from each experimental group were simply processed directly for the localization of peroxidase without incubation with the antisera.

The staining of the nuclei in the same Petri dish was done by indirect immunofluorescence procedure using sera from patients containing antinuclear antibodies (17) (kindly provided by the Department of Immunology of Le Centre Hospitalier de l'Université de Laval). Observations were performed with a Leitz microscope suitable for fluorescence and phase-contrast microscopy. A given field was first observed for fluorescence using a mercury lamp source, the fluorescing nuclei were counted (Fig. 3). Then, the mercury lamp source was shut off using the built-in shutter, and a usual tungsten lamp light source was used and the number of positive cells, stained dark brown by the peroxidase localization reaction, was counted. In some cases, the same field was also observed by phase-contrast microscopy. A total of ~800 cells was counted in each Petri dish in not less than three Petri dishes for each group in most cases.

Scanning Electron Microscopy (SEM)

Petri dishes that were removed from the 37°C incubator were quickly rinsed three times with 0.1 M cacodylate buffer containing 5% sucrose, pH 7.4, pre- 

warmed at 37°C before fixation in situ for 1 h at 37°C with 0.15 M Na-cacodylate containing 1% glutaraldehyde (390 mosmol, pH 7.4) also prewarmed at 37°C. Petri dishes were subsequently rinsed with the cacodylate buffer, postfixed with 1% OsO₄ in 0.1 M Na-cacodylate (pH 7.4), and then dehydrated with increasing concentrations of ethanol. Samples were critical-point dried in a Sandri PVT3 critical-point drying apparatus (Tousimis Research Co., Rockville, Md.) using liquid CO₂ and coated with an ~200-Å layer of gold-palladium alloy in a Hummer II sputter (Technics Inc., Alexandria, Va.). The observations were performed in an ETEC Autoscan U1 scanning electron microscope and sometimes in a JEOL 100CX with ASID attachment. Cells were observed at random (at least 40 per sample) and photographed at a 2,000-6,000 magnification and also at higher resolution (~20,000). To ensure objective observation, the samples were identified only by numbers, the treatments being unknown to the observer.

Transmission Electron Microscopy (TEM)

Petri dishes were quickly rinsed with 0.1 M Na-cacodylate buffer containing 5% sucrose (pH 7.4), and the cells were fixed with 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer for 1 h at 22°C before being scrapped off the bottom of the Petri dishes with a rubber policeman, centrifuged into pellets, rinsed in the buffer, postfixed with OsO₄ in 0.1 M Na-cacodylate (pH 7.4), and conventionally dehydrated in ethanol and embedded in Araldite. Ultrathin sections prepared in a Reichert ultramicrotome were poststained with uranyl acetate and lead citrate and observed in a Siemens Elmiskop 102 electron microscope operated at 60 kV.

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Ultrathin sections were also prepared from cell pellets embedded in Araldite and either osmicated or not and used for the immunocytochemical localization of prolactin according to Stemberger (36). Acid phosphatase, a known marker enzyme for lysosomes, was localized by incubating glutaraldehyde-fixed cell pellets in the medium described by Leduc et al. (28) with one major modification: the substrates consisted of 10 mg of α-glycerophosphate and 10 mg of β-glycerophosphate/10 ml of incubation medium.

RESULTS

Prolactin Secretion

EFFECTS OF SHORT-TERM INCUBATION WITH CB-154: As illustrated in Fig. 1, CB-154 is a highly potent inhibitor of prolactin release, a 93–95% inhibition of basal prolactin secretion being observed up to 16 h of incubation in the presence of the dopamine agonist. In the same experiments, we also measured the cell prolactin content to establish a correlation with the immunocytochemical data. Up to 24 h of incubation, the cell prolactin content was slightly increased in CB-154-treated cells (P < 0.05).

EFFECT OF LONG-TERM TREATMENT WITH CB-154 AND MODULATION BY E2: In anterior pituitary cells maintained in culture for 12 d and treated with CB-154 (10⁻⁶ M) during the last 8 d (Fig. 2 A), there is a marked inhibition of prolactin release in the presence of CB-154 up to 4 d of treatment (P < 0.01). When the treatment is prolonged, however, the inhibition by CB-154 on basal prolactin release is attenuated. In contrast, cells treated with E2 show a marked stimulation of basal prolactin release which increases progressively up to 4 d of treatment and remains stable up to the last interval studied (day 8 of estrogen treatment). In cells treated with CB-154 and E2, the pattern of prolactin release resembles that of E2 alone (Fig. 2 A). Moreover, as illustrated by the 50 and 80% reversals of the inhibitory effects of CB-154 on prolactin secretion on days 2 and 4, respectively, E2 exerts a potent antidopaminergic action directly at the pituitary level. It can be seen in Fig. 2 B that the patterns of the effects of CB-154 and E2 are similar in the presence of 3 nM TRH during the 4-h incubation period although the magnitude of prolactin release is greater.

When prolactin cell content was measured at the end of the 4-h incubation period, CB-154 led to an increased cell content proportional to the inhibition of hormone release, whereas E2 increased cell content from days 4 to 8 (100% above control at day 8) (data not shown). The total prolactin calculated by addition of prolactin released and prolactin cell content is shown in Fig. 2 C and D. It can be seen that E2 increased significantly (P < 0.05) the total prolactin amount already at 2 d, and the amount was increased further at 8 d (P < 0.01). The presence of CB-154 did not significantly modify the stimulatory effect of E2 on total prolactin at any time interval.

IMMUNOCYTOCHEMISTRY: Fig. 3 b illustrates the detection of nuclei by indirect immunofluorescence. Note that there are two types of nuclei: those that are small and round and those that are larger and sometimes more elongated. This technique is reproducible and easily performed and clearly reveals almost all nuclei present. By phase-contrast microscopy (Fig. 3 c) of the same field we can distinguish two types of cells based on nuclear morphology, the "small nuclei" cells are more refringent, dense and round and the "large nuclei" cells are the typically flattened and squamous fibroblasts already described (2). When immunoperoxidase was performed, only the small nuclei cells were positive for prolactin (Fig. 3 a). In parallel experiments we have shown that the same is true for the other five pituitary cell types (TSH, growth hormone [GH], ACTH, follicle-stimulating hormone [FSH], and luteinizing hormone [LH]). In the control preparations described in Materials and Methods, no staining was observed in any of the cells thus demonstrating the specificity of the reaction. A micrograph of such a control has been published elsewhere (4). The other five
FIGURE 3 Simultaneous localization of prolactin by immunoperoxidase (a) and the nuclei of the same cell preparation by immunofluorescence (b) in anterior pituitary cells in primary culture (see text). The same group of cells is also shown by phase-contrast microscopy (c). Note that prolactin-positive cells (thin arrows) densely stained by the reaction products have small nuclei whereas fibroblasts have a characteristic larger size nucleus (fat arrows). The percentage of prolactin-positive cells was always expressed with respect to the total number of small nuclei. In this manner it was possible to exclude fibroblasts from the counts. This immunofluorescent detection of nuclei was also necessary to determine exactly the number of nuclei present. This method was reproducible and gave consistent results in four different experiments. In this experiment, fixation was performed with 4% paraformaldehyde. Bar, 10 μm.
cell types are not shown in this report but all of them could be detected. Prolactin-positive cells, expressed with respect to the number of secretory cells, represented ~70–75% of the total secretory cell population and because they are most numerous, we focused our attention on them. On the other hand, LH cells are ~2%, FSH 4–6%, ACTH 4–8%, TSH 1–3%, GH, 10–18%. Both fixation methods (Bouin and paraformaldehyde) resulted in satisfactory immunocytochemical reactions and have already been described (4). We prefer the latter method because it resulted in more intense immunofluorescence. In six different experiments, consistent results were obtained with both fixation methods with respect to the number of prolactin-positive cells and the relative intensity of the reactions.

**Effects of Various Agents on Prolactin Staining and Cell Number:** 4 h after addition of CB-154, we could already note an increase of the immunocytochemical reaction for prolactin. This intensity of the immunoreactivity slightly increased up to 1 d of treatment with CB-154 (Fig. 4). 8 d after the addition of CB-154, the immunoreactivity of the prolactin-secreting cells was somewhat decreased (see Table I) whereas the control cells remained unchanged. In E2-treated cells, on the contrary, we noted a marked increase of

![Figure 4](image-url)

**Figure 4** Immunocytochemical localization of prolactin in cultured anterior pituitary cells. (a) control (untreated) cells: note moderate reaction in the majority of cells. Negative cells can also be observed. (b) Cells treated for 24 h with CB-154. Note some increase in the intensity of immunoreactivity. Most cells are filled with reaction products. Both preparations were processed, photographed, and micrographs printed in identical conditions. Fixation was performed with Bouin's fixative. (a and b) Bar, 10 μm.
the immunocytochemical reaction and cell hypertrophy (Fig. 5); a semiquantitative evaluation of the reaction is shown in Table I. At day 4 of E2 treatment, the reaction was most intense (++++) whereas that of control was (++). When cells were treated with both E2 and CB-154, the intensity and type of immunocytochemical reaction looked almost superimposable to that of the E2-treated cells although it generally appeared slightly less intense than in the E2-treated cells. E2-induced cell hypertrophy was not affected by CB-154. When E2-pretreated cells were incubated for 4 h with 3 x 10^-9 M TRH before fixation, the intensity of the reaction slightly decreased as compared to E2 alone-treated cells (data not shown). During the course of the study, we have found no change in prolactin cell number (Table II). No effect of E2 or CB-154 was found on prolactin cell number.

**SEM**

**SURFACE MORPHOLOGY OF CONTROL CELLS:** From days 6 to 12 in culture, the cells are firmly attached to the plastic substratum via fiber-like structures. The cells are round (1) and preferentially in clones, although single cells are also seen. Flattened and squamous fibroblasts are easily recognizable underlying the glandular cells. At high resolution, the glandular cells are covered to varying degrees with finger-like projections (microvilli) of 0.1 μm in diameter and more round structures (blebs) 0.1-2 μm (Fig. 6a).

**EFFECT OF ESTRADIOL AND TRH:** When the cells were incubated in the presence of E2, a marked prominent feature was an increase in the microvilli. Sometimes an increase in the blebs was noted. Although of smaller magnitude, incubation with TRH alone led also to an increase in microvilli (Fig. 6b) on many cells. However, when the cells were preincubated with E2 before incubation with TRH for 4 h, the microvilli were increased in prominence and number to a higher degree than seen in the case of either E2 or TRH alone (Fig. 7a). In cells treated with E2 for more than 4 d, there was generally an increase in cell size (hypertrophy).

**EFFECT OF CB-154:** After 4 h in the presence of CB-154, many cells already showed folds and blebs to varying degrees. More easily identifiable morphological alterations could be seen after 12 and 16 h of CB-154 treatment: many cells flattened slightly or presented irregular or elongated form. A noticeable feature was the apparent decrease in the number and sometimes even the disappearance of microvilli (Fig. 7b). Some cells were to be devoid of surface protrusions except occasional microvilli or blebs. This was accompanied by a decrease of the cell size as though cell retraction had occurred. Long-term treatment of the cells with CB-154 up to 8 d did not lead to further modifications of the features observed at 12-16 h except the

| Days of incubation | 1   | 2   | 4   | 6   | 8   |
|-------------------|-----|-----|-----|-----|-----|
| Control           | ++  | ++  | ++  | ++  | ++  |
| CB-154            | +++ | ++  | +++ | +   | +   |
| E2                | ++  | +++ | +++ | +++ | +++ |
| CB-154 + E2       | +++ | +++ | +++ | +++ | +++ |

Evaluation of the intensity of the reaction by two observers. For the corresponding micrographs, see Figs. 4 and 5. For the corresponding radioimmunoassay curves, see Figs. 1 and 2.

**TABLE II**

| Time in culture | Control | E2  | Control | E2  | Control | E2  |
|-----------------|---------|-----|---------|-----|---------|-----|
| No. of secretory cells | 802    | 725 | 622    | 617 | 791    | 726 |
| No. of prolactin cells | 601    | 553 | 453    | 465 | 568    | 503 |
| % of prolactin cells | 75     | 76  | 73     | 75  | 72     | 69  |
| No. of fibroblasts | 56     | 50  | 178    | 184 | 459    | 386 |

After 3, 6, or 12 d in culture, in the presence or absence (control) of E2, simultaneous staining of prolactin and the nuclei was performed as described under Materials and Methods. The reported numbers are cell counts of 20 constant microscope fields at x40 objective lens. Percentages of prolactin-positive cells were calculated with respect to the number of secretory cells and not the total number of cells (secretory and fibroblasts) because the number of fibroblasts increased with time.

**FIGURE 5** Immunocytochemical localization of prolactin in control cells (those that received the vehicle alone) (a), and E2-treated cells for 6 d (b). These micrographs demonstrate the E2-induced cell hypertrophy of prolactin cells. Note that most prolactin cells are hypertrophied and densely stained in (b) as compared to those in (a). The cell nuclei in both experimental conditions have comparable sizes. Both cell preparations were fixed, processed, photographed, and micrographs printed in identical conditions. Fixation was performed, in this experiment, with 4% paraformaldehyde. (a and b) Bar, 10 μm.
FIGURE 6 Scanning (a and b) and transmission electron microscopy of cultured pituitary cells incubated for 4 h with 3 nM TRH. (a) control (untreated) cell. Note occasional microvilli (arrow) and blebs (b). Bar, 10 µm. (b) Cell treated with TRH. Note the increased number of microvilli (arrow). Bar, 1 µm. (c) Ultrathin section of TRH-treated cell showing the blebs (b) and some microvilli. Pleomorphic prolactin secretory granules are seen (sg) but the cell is significantly degranulated as compared to controls (cf. Fig. 8). Small vesicles (v) are seen near the cell membrane at various locations. × 15,000.

appearance of a more irregular and folded cell surface and presence of large blebs on some cells.

EFFECTS OF SIMULTANEOUS TREATMENT WITH E2 AND CB-154: Generally, the appearance of cells treated with E2 and CB-154 was similar to that of cells treated with E2 alone with the prominence of many microvilli and blebs. There was also a significant hypertrophy of many cells.

Before revealing the identities of the samples to the observer, he was asked to attempt assessing the samples' treatment on the basis of the surface features described above. In three different experiments, the observer was able to guess correctly among the following samples: untreated vs. E2; untreated vs. CB-154; untreated vs. TRH, untreated vs. E2 and TRH; and CB-154 vs. E2.

TEM

Because prolactin cells represent ~70–75% of the total of glandular cells, we focused our observations on them. TSH-secreting cells which are only 1–3% in number were seldom encountered in ultrathin sections. Prolactin cells in culture displayed most of the characteristics observed in vivo, namely the typical pleomorphic secretory granules. A prominent Golgi complex was often noted. When the immunocytochemical

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Localization of prolactin was performed, the secretory granules were densely stained (Fig. 8).

Effect of E2 and CB-154 on the Ultrastructure (TEM) of Prolactin-Secreting Cells: In most cells treated with E2 up to 6 d, we noted an increase in the Golgi elements and Golgi vesicles, immature granules, and rough endoplasmic reticulum (Fig. 9). Moreover, the saccules of Golgi were often distended and hypertrophied. In general, large secretory granules were seldomly seen up to 4 d of treatment but could be seen in some cells between days 4 and 8. The nucleolus was often prominent. In cells treated with CB-154 from 4 to 24 h (short term), secretory granules seemed to

Figure 7 Effect of E2 and TRH vs. CB-154 on the cell surface of pituitary cells in primary culture. (a) This cell was pretreated with E2 for 4 d and finally stimulated with TRH for 4 h before fixation; note the microvilli which cover the cell. (b) A representative cell that has been treated with CB-154 for 4 d, note absence of microvilli from the surface; numerous blebs are present. Although a micrograph of control (untreated or treated with the vehicle alone) from this experiment is not shown it is comparable to that of another experiment (Fig. 6 a). Bars, 1 µm.

Figure 8 Immunocytochemical localization of prolactin directly on an ultrathin section of glutaraldehyde-osmium fixed and Araldite-embedded control cells. Prolactin secretory granules (sg) are densely stained. Note however a variation in staining intensity among sg population. This micrograph serves two purposes: first, the identification of a prolactin cell and secondly, the demonstration of a good ultrastructural preservation associated with immunocytochemistry. Lysosomes (L) are contrasted but show no immunocytochemical reaction. Note well-developed Golgi (G). × 20,000.
accumulate in the cells, most of these granules being larger than in control or E2-treated cells (Fig. 10). Some granules occupied large areas of the cells and were usually separated from the cell membrane by a few layers of rough endoplasmic reticulum (Fig. 10). Golgi saccules and Golgi vesicles were still observed although in smaller numbers than in controls. Dense bodies, probably of lysosomal origin (because they are acid phosphatase positive), and autophagic vacuoles were seen in most cells although their number varied from cell to cell (Fig. 10). After long-term treatment of the cells with CB-154 (2-8 d), we observed, in most cells, secretory granules even larger than those seen at earlier stages. In some cases, these granules were surrounded completely by layers of endoplasmic reticulum.

EFFECT OF TRH ON THE ULTRASTRUCTURE (TEM) OF THE PROLACTIN CELLS: After 1 h of treatment of the cells with TRH, we observed in some cells a distribution of the granules along the cell membrane whereas after 4 h in the presence of TRH most cells were significantly degranulated (Fig. 6c). Many small vesicles were seen near the cell membrane at various locations (Fig. 6c). The sections where the microvilli and blebs were cut showed that the content of blebs is similar to the cell “cytoplasm” (Fig. 6c).

DISCUSSION
The present data give, for the first time, morphological evidence for the stimulatory effect of E2 and TRH on prolactin cells in primary culture and describe the cellular changes induced by the potent dopamine agonist, CB-154. It was also possible to show that the action of CB-154 on cellular morphology could be reversed by E2, thus giving further support to the original observations of Raymond et al. (34) showing the antidopaminergic action of E2 on prolactin secretion in pituitary cells in culture. The results obtained by scanning and transmission electron microscopy and by immunocytochemistry are in good agreement with the radioimmunoassay data of the same cell preparation. In fact, prolactin secretion is markedly inhibited by short-term treatment with CB-154. Because the cell content of prolactin is proportionally increased, the total amount of prolactin in the cultures is only slightly affected indicating that release rather than synthesis has been affected at short-term intervals. The accumulation of prolactin in the cells is visualized by an increase in the immunocytochemical reaction. Moreover, in agreement with the light microscope findings, the TEM shows a characteristic accumulation of secretory granules (Fig. 9). The present observations resemble those noted after treatment of similar pituitary cells in culture with 10^{-7} M colchicine for 4-24 h (3). With short-term CB-154 treatment, the endoplasmic reticulum and the Golgi are not significantly affected.

As observed previously (1-3), SEM also gives an indication of the secretory state of cells in culture. Marked increases or decreases of the microvilli seem to accompany increases or decreases in hormone secretion, respectively. These observations give more support to the findings of Centola (12) who...
showed a correlation between progesterone secretion by rat granulosa cell cultures and the number of microvilli on the cell surface. In addition our data, with respect to the effect of TRH on the cell surface or prolactin cells, are in agreement with a recent TEM study (8). It thus appears that the modifications of the cell surface are closely related to the secretion processes (1–3, 9, 12). In this context, various reports show that transformed cells, that are known to be more active than their normal counterparts, show a rough surface consisting of microvilli and blebs (23, 32). The diameter of microvilli in this study as well as previous reports (1–3, 23, 32) is the same: ~0.1 μm.

The maximal effects of E2 are between 4 and 8 d as evidenced by both radioimmunoassay and morphological data. We could not extend the experiments for longer periods because, in preliminary studies, the cells treated with CB-154 for more than 8 d showed marked signs of regression as observed by electron microscopy. However, the stimulatory effect of E2 in these cells remains maximal up to at least 3 wk in culture (27).

In this context, Graf et al. (21), in a light microscope study, demonstrated that the prolactin inhibitory agents, CB-154 and bisuride hydrogen bromide, could induce cell regression. E2, on the contrary, is shown in this study to induce cell hypertrophy of most of the cultured cells. Estrogens have also been shown to induce hypertrophy and hyperplasia of prolactin cells in vivo (5, 20). During the course of the study, we have found no change in prolactin cell number. Therefore, total prolactin cell number is the only meaningful value to be used because total protein and/or DNA can be (and are) markedly affected by fibroblasts. Our results are at variance with the observations of Baker et al. (6) who, although they performed no cell counts, concluded that prolactin cells “proliferate” in culture.

Treatment with CB-154 up to 24 h resulted in an increased intensity of the immunocytochemical reaction for prolactin. At later stages, the reaction decreased. This is in full agreement with the TEM observations where accumulated granules were seen in large number up to 24 h, this effect being followed by a decrease in the number of granules at later time intervals in the presence of CB-154. The changes of cell content of radioimmunoassayable prolactin are also in agreement with these observations. This decrease in the hormone content after long-term incubation with CB-154 can be explained by the appearance of lysosomes and autophagic vacuoles, a known cellular process which eliminates accumulated secretory granules (18).

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