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Bromoergocryptine-Induced Prolactin Degradation in Cultured Pituitary Cells†

Richard A. Maurer

ABSTRACT: Continuous labeling of pituitary cells with [^{3}H]-leucine resulted in a linear accumulation of [^{3}H]prolactin in control cultures, but in bromoergocryptine (CB-154) treated cultures the rate of [^{3}H]prolactin accumulation decreased with time. The possibility that this decreased accumulation of labeled prolactin was due to CB-154-induced prolactin degradation was examined by incubating cells for 30 min with [^{3}H]leucine followed by incubation in a chase medium containing a 400-fold excess of unlabeled leucine. In control cultures, there was little degradation of [^{3}H]prolactin over a 24-h period. In cultures containing CB-154 in the chase incubation medium, there was a 22% decrease in labeled prolactin after 8 h and a 50% decrease after 24 h. Pretreatment of cells with CB-154 for 24 h before pulse-chase analysis resulted in a greater rate of prolactin degradation than was observed in cells treated with CB-154 during the chase incubation only. CB-154 treatment did not affect the degradation of nonprolactin proteins, demonstrating the specificity of its effects. Cycloheximide did not affect prolactin degradation in CB-154-pretreated cells; however, cycloheximide blocked the ability of CB-154 to induce prolactin degradation when the two drugs were added simultaneously. The relationship between prolactin synthesis and degradation was examined in cells treated for varying times with CB-154 and then pulsed for 30 min with [^{3}H]leucine followed by a 4-h chase incubation. Prolactin synthesis declined sharply after 1–2 days of CB-154 treatment and reached a new plateau of 22% of control values after 4 days of treatment. Prolactin degradation was maximal after 1 day of CB-154 treatment and returned toward control values after 3–4 days of treatment. Lysosomes are likely involved in CB-154-induced prolactin degradation as chloroquine is able to partially block CB-154 effects. These studies suggest that CB-154 is able to induce substantial prolactin degradation. Thus, prolactin degradation is involved in removal of excess prolactin which accumulates in the pituitary when prolactin secretion is inhibited.

The rate of production of a protein is dependent on the rate of synthesis and the rate of degradation. A large body of research has shown that mammalian cells continuously synthesize, degrade, and resynthesize most cellular proteins (Goldberg & Dice, 1974; Bradley & Schimke, 1975). Although the degradation of cellular proteins has been extensively studied, relatively little is known about the degradation of secretory proteins such as the pituitary hormone prolactin. The ultrastructural studies of Smith & Farquhar (1966) have demonstrated that when prolactin secretion is greatly inhibited, prolactin secretory granules are incorporated into lysosomes. This finding clearly suggests that when prolactin secretion is blocked, excess hormone is degraded. There have been very few biochemical studies of prolactin degradation. Dannies & Tashjian (1973) used pulse-chase studies to examine prolactin degradation by GH_3 pituitary tumor cells. They found no evidence for any degradation of prolactin under the conditions they examined. Thus, although the electron microscopy study of Smith and Farquhar clearly suggested a role for prolactin degradation in the regulation of prolactin levels, there has been no biochemical evaluation of this process.

In the present study, prolactin degradation has been examined in monolayer cultures of dispersed pituitary cells. In particular, the effects of the potent dopaminergic drug 2-bromo-α-ergocryptine (CB-154) on the degradation of prolactin have been studied. Several studies have shown that dopamine and dopamine agonists can block prolactin secretion (MacLeod et al., 1970; Birge et al., 1970; Caron et al., 1978). Furthermore, the observation that dopamine levels in hypophyseal portal blood vary inversely with prolactin secretion (Ben-Jonathan et al., 1977) suggests that dopamine is likely the physiological prolactin-inhibitory factor. The present experiments examined the effects of CB-154 on prolactin degradation by using continuous and pulse-chase labeling studies. In addition, the effects of the lysosomal stabilizer

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chloroquine on prolactin degradation were examined.

Materials and Methods

Materials. L-[4,5]Leucine (50 Ci/mmol) and NCS tissue solubilizer were purchased from Amersham Corp. (Arlington Heights, IL). Chloroquine diphosphate was purchased from Sigma (St. Louis, MO). Minimal essential medium (MEM) with D-valine substituted for L-valine (MEM-D-valine), Dulbecco's modified Eagle's medium (DMEM), minimal essential medium with leucine omitted, and fetal calf serum were from Gibco (Grand Island, NY). [^{14}C]Prolactin was prepared as described previously (Maurer et al., 1976). Goat antisera to rat prolactin were a generous gift of Dr. Mara Lieberman (University of Wisconsin). 2-Bromo-α-ergocryptine was a gift of Sandoz, Inc. (East Hanover, NJ).

Pituitary Cell Culture. Pituitaries from female, retired breeder rats (Holtzman) were dispersed by treatment with 0.3% collagenase and 0.25% viokase as described by Vale et al. (1972). Dispersed cells were added to 35-mm tissue culture dishes in 2 mL of MEM-D-valine supplemented with 5% dialyzed fetal calf serum, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) (pH 7.4), 100 units/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL fungizone. The D-valine culture medium was used as previous studies have shown that this medium limits fibroblast proliferation (Gilbert & Migeon, 1975). Also, initial experiments demonstrated that pituitary cultures producing large amounts of prolactin were established in this medium. Cells were plated at a density of (1–2) $\times$ 10^6 per dish and were maintained at 37 °C in an atmosphere of 5% CO_2 and 95% air. After 2 days, the medium was replaced with DMEM supplemented with 20 mM Hepes (pH 7.4) and the same antibiotics as above. Cells were maintained for 1–2 days in DMEM before use in experiments.

Pulse-Chase Labeling. The medium was removed, and the cells were rinsed by addition of 1 mL of Earle's balanced salt solution which was removed after 1–2 min. Then 0.5 mL of MEM containing 25 μM leucine and 20 μCi/mL [^{3}H]leucine was added, and the cultures were incubated for 30 min at 37 °C. After incubation, the labeling medium was removed and replaced with 0.5 mL of chase medium which consisted of MEM containing 10 mM leucine. The cells were incubated in the chase medium for varying times at 37 °C. Incubations were stopped by removal of chase medium and addition of 0.3 mL of an ice-cold solution of 10 mM sodium phosphate (pH 7.4), 0.15 M NaCl, 10 mM leucine, 1% Triton X-100, and 0.5% deoxycholate. The cells were removed from the culture dish in this solution and then homogenized in a Teflon glass homogenizer. The homogenate was centrifuged at 10000g for 10 min prior to immunoprecipitation.

Immunoprecipitation. Aliquots of the cell extract and the incubation medium were combined with 2.5 μg of [^{14}C]prolactin and an amount of antiprolactin in excess of that required to precipitate the added carrier [^{14}C]prolactin (Maurer et al., 1976). After incubation overnight at 4 °C, the immunoprecipitate was washed, pelleted, and prepared for scintillation counting as described previously (Maurer, 1979; Maurer & Gorski, 1977). This immunoprecipitation procedure has previously been shown to be specific for prolactin (Maurer et al., 1976; Maurer & Gorski, 1977). Data from immunoprecipitations were corrected for the recovery of the [^{14}C]prolactin. In most experiments, 75–100% of the [^{14}C]prolactin added to the sample was recovered in the immunoprecipitate. For analysis by gel electrophoresis, immunoprecipitates were dissolved in 1% sodium dodecyl sulfate, 4 M urea, 10 mM Tris (pH 7.4), and 1% β-mercaptoethanol and then heated in a boiling water bath for 5 min. Samples were electrophoresed on sodium dodecyl sulfate containing 10% polyacrylamide gels by using the buffer system described by Laemmli (1970). After electrophoresis, the gels were sliced, and each slice was incubated with 0.5 mL of NCS for 2 h at 45 °C prior to addition of scintillation fluid.

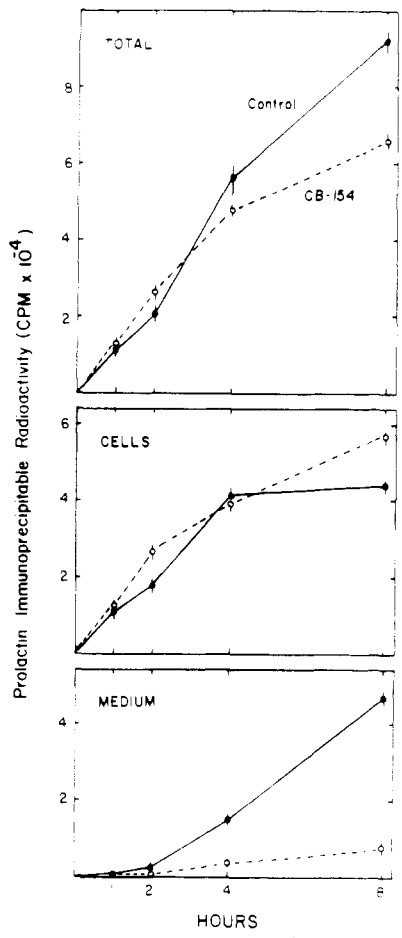

FIGURE 1: Time course of incorporation of [^{3}H]leucine into prolactin in control cultures and cultures incubated with 10 nM CB-154. Control pituitary cell cultures (●) or cultures containing 10 nM CB-154 (○) were incubated for 1, 2, 4, or 8 h in MEM containing 0.2 mM leucine and 20 μCi/mL [^{3}H]leucine. After the incubations, the amount of radioactivity incorporated into prolactin was determined for aliquots of the cell homogenates and the medium by specific immunoprecipitation as described under Materials and Methods. The total incorporation of radioactivity into prolactin was determined by addition of the amount of radioactive prolactin in the cells and in the medium. Values are means ± standard error of the mean for three independent determinations per point.

Results

The effects of CB-154 on prolactin accumulation were investigated in a continuous-labeling experiment (Figure 1). In control cells, labeled prolactin first accumulated in the cells. After a lag of about 2 h, labeled prolactin began to accumulate in the medium. In CB-154-treated cells, labeled prolactin accumulated in the cells, but very little was secreted into the medium. In controls, the total amount of labeled prolactin in the system (cells + medium) increased in an approximately linear fashion. However, in the CB-154-treated cultures, the rate of labeled prolactin accumulation appeared to decrease with time. The nonlinear accumulation of labeled prolactin in CB-154-treated cultures might be due to effects on prolactin synthesis or on prolactin degradation.

Pituitary cells were first pulse labeled with [^{3}H]leucine to investigate the possibility that CB-154 induces prolactin

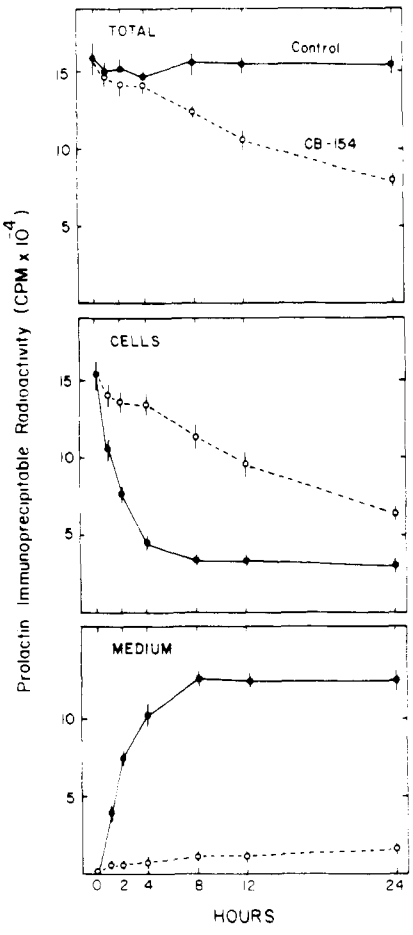

FIGURE 2: Time course of degradation of newly synthesized prolactin in control cultures and cultures containing 10 nM CB-154. Pituitary cell cultures were labeled for 30 min in MEM containing 25 μM leucine and 20 μCi/mL [^{3}H]leucine in either the absence (●) or the presence (○) of 10 nM CB-154. After the pulse labeling, the medium was removed and replaced with chase medium consisting of MEM containing 10 mM leucine. Cultures which were labeled in medium containing CB-154 also were treated with CB-154 during the chase incubation. At 0, 1, 2, 4, 8, 12, and 24 h after labeling, aliquots of cell homogenates and medium were analyzed for radioactive prolactin by immunoprecipitation. The values are means ± standard error of the mean for three determinations per point. The values for control cultures and CB-154-treated cultures overlap each other for the 0-h chase time.

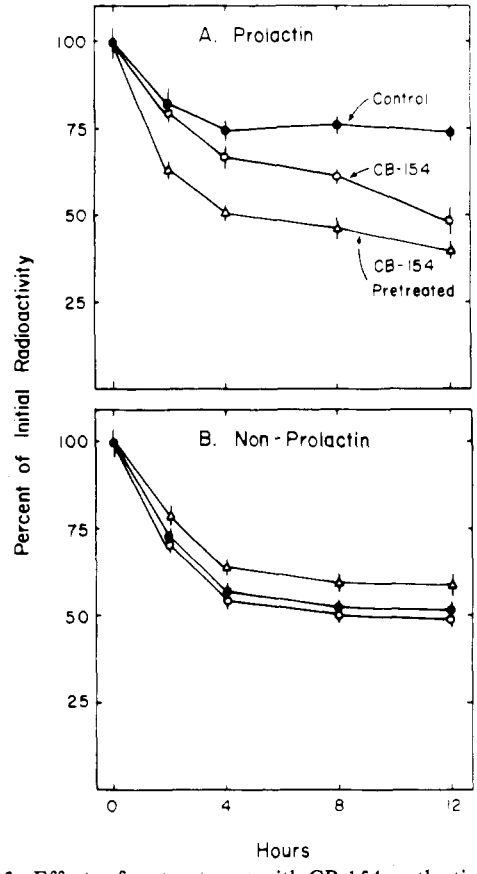

FIGURE 3: Effects of pretreatment with CB-154 on the time course of the degradation of prolactin and nonprolactin proteins. Pituitary cell cultures were pulse labeled for 30 min in MEM containing 20 μCi/mL [^{3}H]leucine and incubated for 0, 2, 4, 8, or 12 h in chase medium. The amount of total labeled prolactin in cells + medium (A) was determined for control cultures (●), for cultures treated with 10 nM CB-154 during the chase incubation only (○), and for cultures treated with CB-154 for 24 h prior to labeling and also during the labeling and chase incubations (△). For each group, the amount of labeled prolactin remaining at each time point is expressed as a percentage of the radioactivity incorporated into prolactin during the 30-min pulse-labeling incubation. The amount of radioactivity in nonprolactin proteins (B) was determined by analysis of total radioactive protein by trichloroacetic acid precipitation and subtraction of the amount of radioactive prolactin for each sample. The data for nonprolactin proteins are also expressed as a percentage of the initial radioactivity. The values are means ± standard error of the mean for three determinations per point.

degradation. The cells were then transferred into chase medium containing 10 mM unlabeled leucine with or without 10 nM CB-154. The 10 mM unlabeled leucine is 400-fold greater than the leucine concentration used for the labeling pulse and helps to stop further incorporation of [^{3}H]leucine during the chase period. In control cultures, prelabeled prolactin was quickly depleted from the cells and accumulated in the medium (Figure 2). The total amount of labeled prolactin in the system (cells + medium) was relatively constant for 24 h, the duration of the experiment. In the cells treated with 10 nM CB-154, the fate of prelabeled prolactin was quite different. The CB-154 very effectively blocked the release of prelabeled prolactin into the medium while the cell content of labeled prolactin gradually declined. Thus, in the CB-154-treated cultures, the total amount of labeled prolactin (cells + medium) decreased gradually over the 24-h duration of the experiment, suggesting the degradation of prolactin. In this study, the amount of labeled prolactin was determined by immunoprecipitation. It seemed possible that during the chase period the prolactin molecule might be partially degraded but still react with the antibody. This possibility was explored by analysis on denaturing sodium dodecyl sulfate containing polyacrylamide gels. Analysis of immunoprecipitates from samples of control cultures or CB-154-treated cultures taken immediately after labeling or after a 24-h incubation in chase medium revealed only a single peak of radioactivity which comigrated with a [^{14}C]prolactin standard (data not shown). This suggests that if any partial degradation products of prolactin remain in the pituitary, they do not contain sufficient antigenic determinants for immunoprecipitation.

The effect of pretreatment with CB-154 on subsequent prolactin degradation was also examined (Figure 3A). In this study, the time course of prolactin degradation was studied in cells pretreated with CB-154 for 24 h prior to the pulse labeling as well as in control cultures and cultures treated with CB-154 during the chase incubation only. CB-154, as well as other dopaminergic drugs, specifically inhibits prolactin synthesis in cultured pituitary cells (R. Maurer, unpublished experiments; see also Figure 4A). Therefore, for each group, the amount of radiolabeled prolactin remaining at any time point is expressed as a percentage of the initial radioactivity

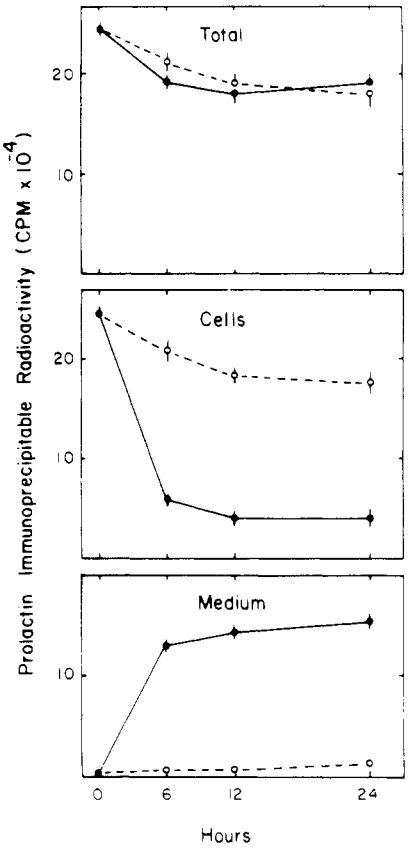

FIGURE 4: Effect of cycloheximide on the time course of CB-154-induced prolactin degradation. Pituitary cell cultures were labeled for 30 min with [^{3}H]leucine and then incubated for 0, 6, 12, or 24 h in medium containing 2 μg/mL cycloheximide and a 400-fold excess of unlabeled leucine in either the absence (●) or the presence (○) of 10 nM CB-154. Radiolabeled prolactin at each time point was determined by immunoprecipitation. The total amount of radiolabeled prolactin was determined by addition of the amount in cells and medium. The values are means ± standard error of the mean for three independent determinations per group.

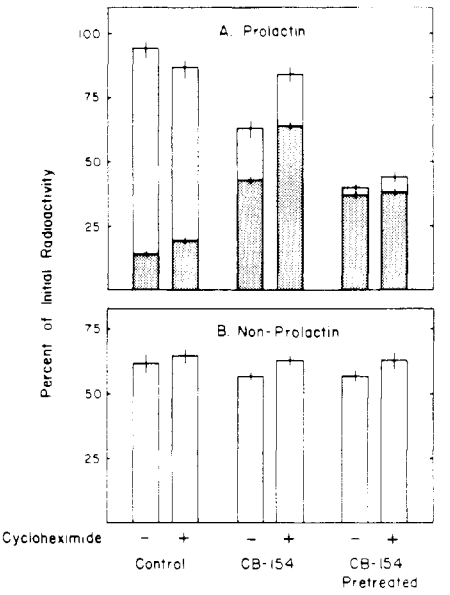

FIGURE 5: Analysis of cycloheximide effects on degradation of prolactin and nonprolactin proteins in control cultures, cultures treated with CB-154 during the chase incubation only, and cultures pretreated with CB-154 for 24 h prior to pulse labeling. Pituitary cells were labeled for 30 min in MEM containing 20 μCi/mL [^{3}H]leucine and then transferred to chase medium containing a 400-fold excess of unlabeled leucine either with or without 2 μg/mL cycloheximide. Control cultures were not treated with CB-154. The CB-154 group received 10 nM CB-154 during the chase incubation only, and the CB-154 pretreatment group was treated with 10 nM CB-154 for 24 h prior to pulse labeling as well as during the labeling and chase incubation. The amount of labeled prolactin (A) was determined by immunoprecipitation for each group. The results are expressed as a percentage of the total amount of radioactivity incorporated into prolactin during the 30-min pulse labeling. The hatched portion of the histogram represents the cell content of labeled prolactin, the open portion represents the labeled prolactin in the medium, and the total height of the histogram indicates the total percentage of labeled prolactin remaining in the system after the 24-h chase incubation. Radioactive nonprolactin proteins remaining after the 24-h chase incubation (B) were determined as in Figure 3 and are expressed as a percentage of the amount of radioactivity incorporated into nonprolactin proteins during the 30-min pulse labeling. Values are means ± standard error for three independent determinations per group.

incorporated into prolactin during the 30-min pulse. The results suggest that cultures preincubated with CB-154 had considerably greater prolactin degradation, especially at early time points after the pulse labeling, than did cultures which were treated with CB-154 during the chase incubation only. Also, in controls, approximately 25% of the labeled prolactin was degraded during the 12-h chase incubation. This is considerably more degradation than was observed in controls in the previous experiment (Figure 2). The reason for this difference in prolactin degradation in controls is not clear. In this experiment, the degradation of radiolabeled nonprolactin proteins was also examined (Figure 3B). The time course of degradation of nonprolactin was very similar in all three groups, demonstrating the specificity of the CB-154 induction of prolactin degradation.

In the previous pulse-chase experiments, cultures were incubated with an excess of unlabeled leucine during the chase incubations in order to prevent further incorporation of [^{3}H]leucine. However, excess unlabeled leucine may not be sufficient to prevent further incorporation, especially if there is preferential reutilization of amino acids from degraded protein. In that case, the pulse-chase studies would underestimate the amount of prolactin degradation. This possibility was explored by using chase incubations containing cycloheximide. Preliminary experiments demonstrated that cycloheximide concentrations of 2 μg/mL blocked 94% of total protein synthesis in cultured pituitary cells. Therefore, pituitary cells were pulsed for 30 min with [^{3}H]leucine and then incubated in chase medium containing unlabeled leucine and 2 μg/mL cycloheximide either with or without CB-154 (Figure 4). Although CB-154 was still able to block prolactin release in this experiment, it did not induce prolactin degradation greater than that observed in controls. This suggests that protein synthesis is required for CB-154-induced prolactin degradation. This was examined further in an experiment examining the effects of cycloheximide on prolactin and nonprolactin protein degradation in control, CB-154-treated, and CB-154-pretreated cultures (Figure 5). In this experiment, as in the previous one, cycloheximide essentially eliminated the ability of CB-154 to induce prolactin degradation when the CB-154 was added simultaneously with the cycloheximide. However, in CB-154-pretreated cells, cycloheximide did not prevent prolactin degradation. This finding suggests that protein synthesis is necessary for the initiation of CB-154-induced prolactin degradation, but it is not necessary to maintain prolactin degradation in pretreated cells. Also, the finding that cycloheximide had little effect on prolactin degradation in the control and pretreated groups or on nonprolactin protein degradation in any of the groups suggests that chase incubations with unlabeled leucine are sufficient to block further incorporation of radioactivity during the chase period.

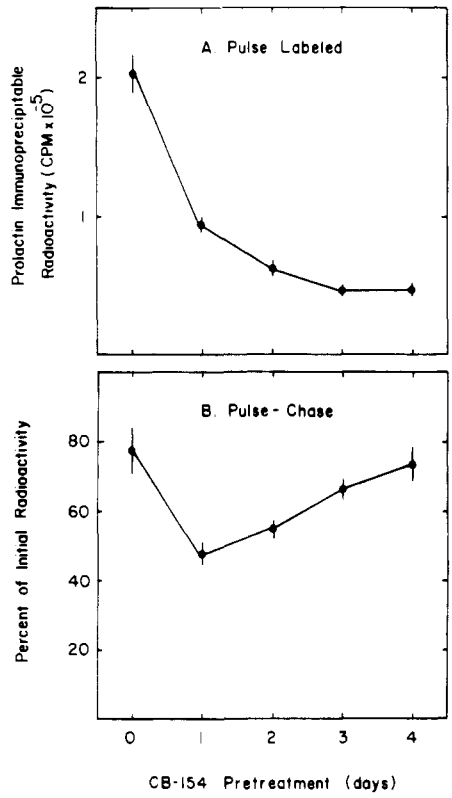

FIGURE 6: Effect of varying periods of CB-154 pretreatment on prolactin synthesis and degradation. Pituitary cells were pretreated for 1, 2, 3, or 4 days with 10 nM CB-154 while control cultures (0 pretreatment) received no CB-154. The cells were labeled for 30 min with [^{3}H]leucine and then incubated in chase medium for 4 h. Treatments were arranged so that all cells were labeled at the same time. Cells pretreated with CB-154 for 1, 2, 3, or 4 days were also treated with CB-154 during the pulse and chase incubations. Prolactin synthesis (A) was estimated by determination of the amount of radioactivity incorporated into prolactin during the 30-min pulse. Prolactin degradation (B) was estimated by determining the percentage of labeled prolactin which remained after the 4-h chase incubation. Values are means ± standard error of the mean for three determinations per point.

The relationship between CB-154-induced effects on prolactin synthesis and prolactin degradation was examined in an experiment which compared the results of pulse-labeling and pulse-chase incubations (Figure 6). Pituitary cells were pretreated with CB-154 for 0, 1, 2, 3, or 4 days. Prolactin synthesis was determined by pulse-labeling cells for 30 min followed by quantitation of newly synthesized prolactin by immunoprecipitation. The results demonstrated that prolactin synthesis decreased sharply after 1–2 days of CB-154 treatment and reached a new plateau of about 22% of control values after 3–4 days of treatment (Figure 6A). Prolactin degradation was estimated by pulse-labeling cells for 30 min followed by a 4-h chase incubation. The 4-h chase incubation was chosen as previous results (Figure 3) showed that this interval was sufficient for substantial prolactin degradation in CB-154-pretreated cells. Prolactin degradation was maximal after 1 day of CB-154 treatment and returned to values similar to those in controls after 3–4 days of treatment (Figure 6B).

In order to investigate the possible role of lysosomes in CB-154-induced prolactin degradation, the effects of chloroquine were investigated. Chloroquine, which has been shown to inhibit lysosomal degradation of protein (Wibo & Poole, 1974; DeDuve et al., 1974), was able to partially inhibit the ability of CB-154 to stimulate prolactin degradation (Figure 7). Chloroquine did not interfere with the ability of CB-154

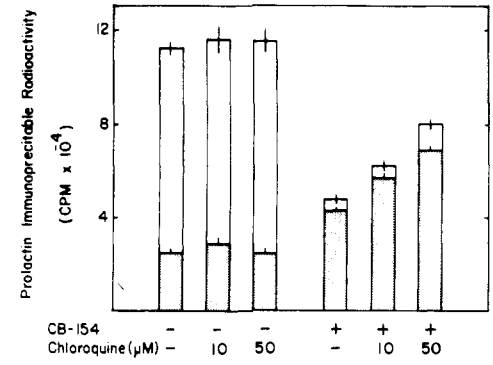

FIGURE 7: Effect of chloroquine on degradation of newly synthesized prolactin in control and CB-154-treated cultures. Pituitary cell cultures were pulsed labeled for 30 min in medium containing 20 μCi/mL [^{3}H]leucine and then incubated for 24 h in chase medium either with or without 10 nM CB-154 and the indicated amount of chloroquine. The amount of radioactive prolactin remaining in the cells (hatched portion of the histogram) and in the medium (open portion of the histogram) was determined by immunoprecipitation. The total height of the histogram indicates the total amount of [^{3}H]prolactin in the system after the 24-h chase period. Values are means ± standard error of the mean for three independent determinations per group.

to block the release of labeled prolactin from the pituitary.

Discussion

The present findings clearly offer biochemical evidence for CB-154-induced prolactin degradation. After 24 h in medium containing 10 nM CB-154, almost half of the prelabeled prolactin was degraded. Pretreatment of pituitary cells with CB-154 for 24 h before pulse labeling resulted in an even more rapid degradation of prolactin. Thus, this mechanism can result in substantial prolactin degradation. In control cultures, prolactin degradation was relatively low but somewhat variable from experiment to experiment. The reason for the variability in prolactin degradation by control cultures is unknown. In all cases, CB-154 induced much more degradation than was found in control cultures. Dannies & Tashjian (1973) found that there was little or no prolactin degradation in GH_3 pituitary tumor cells.

The ability of CB-154 to induce prolactin degradation is likely related to the ability of this drug to block prolactin release. Thus, retention of prolactin in the cell appears to make it susceptible to degradation. However, retention of prolactin in the cell does not automatically lead to its degradation. For instance, in control cells prelabeled prolactin is rapidly depleted from the cells during the chase, but secretion slows down and reaches a plateau after about 8 h, leaving about 25% of the prelabeled hormone retained in the cell. This pattern of prolactin release in consistent with the suggestion that there may be two pools of prolactin, a quickly released pool and a more slowly released pool (Swearingen, 1971). Although prelabeled prolactin is retained in the control cells for up to 24 h, there appears to be little degradation. It may be that the total amount of hormone retained in the tissue is the stimulus which triggers degradation. Alternatively, it may be that degradation is stimulated by an action of CB-154 independent of the effect on prolactin secretion.

Simultaneous addition of cycloheximide and CB-154 to chase incubation medium prevented CB-154-induced prolactin degradation, although CB-154 was still able to inhibit prolactin release. Cycloheximide did not block CB-154-induced prolactin degradation in cells pretreated with CB-154. This suggests that protein synthesis is required to initiate but not to maintain CB-154-induced prolactin degradation. Cycloheximide has been found to block the degradation of cellular proteins induced by serum deprivation (Hershko & Tomkins,

1971). Amenta et al. (1977) have suggested that the ability of cycloheximide to block protein degradation is due to effects on the levels of lysosomal proteases. The present finding that cycloheximide prevents the initiation but not the maintenance of CB-154-induced prolactin degradation suggests the possibility that the cycloheximide effects involve proteins other than lysosomal proteases.

CB-154 affects both prolactin synthesis and degradation. Prolactin synthesis decreased sharply after 1–2 days of CB-154 treatment and apparently reached a new plateau value after 3–4 days of treatment. The time course of CB-154 effects on prolactin degradation was quite different, with degradation being maximal after 1 day of treatment and returning toward control values after 3–4 days of treatment. This time course of CB-154 effects on prolactin degradation probably reflects the fact that CB-154 immediately inhibits prolactin release, but several days are required for its full effects to inhibit prolactin synthesis. Thus, prolactin accumulates at early time points after CB-154 treatment, and degradation allows the removal of excess prolactin. After 3–4 days of CB-154 treatment, prolactin synthesis has decreased considerably, which would lead to less prolactin accumulation and less prolactin degradation.

In a continuous-labeling experiment, the amount of newly synthesized prolactin which accumulated in 8 h was reduced in CB-154-treated cultures. Although the effects of CB-154 on prolactin synthesis have not been investigated at early time points, the large inhibition of prolactin synthesis observed after 24 h of treatment suggests the possibility of significant inhibition of prolactin synthesis after 8 h. Also, the observed rate of CB-154-induced prolactin degradation is probably not sufficient to completely account for the reduced prolactin accumulation observed after 8 h. Thus, it seems quite likely that the reduced accumulation of newly synthesized prolactin is due to CB-154 effects on both the synthesis and degradation of prolactin.

The electron microscopic studies of Smith & Farquhar (1966) suggested that lysosomes were involved in prolactin degradation. The present study using chloroquine offers biochemical evidence supporting this view. Chloroquine accumulates in lysosomes and inactivates lysosomal enzymes (Lie & Schofield, 1973; DeDuve et al., 1974; Carpenter & Cohen, 1976; Wibo & Poole, 1974). Thus, the ability of chloroquine to partially inhibit CB-154-induced prolactin degradation is consistent with the involvement of lysosomes in this process.

The degradation of prolactin would seem to be a wasteful mechanism for the regulation of intracellular prolactin content. This degradatory mechanism is probably necessary due to the fact that prolactin mRNA likely has a long half-life and, therefore, prolactin synthesis cannot change very rapidly (Stone et al., 1977). Thus, although prolactin secretion can be very rapidly inhibited by dopamine and dopamine agonists, the rate of prolactin synthesis cannot change as rapidly. This leads to accumulation of prolactin in the pituitary when prolactin secretion is blocked. It seems clear that the lysosomal degradation allows for the removal of this excess prolactin.

Acknowledgments

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