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Effects of Culture Age on PRL and GH Responses to Bromocriptine and Somatostatin from Primary Cultures of Rat Anterior Pituitary Cells (41470)

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Abstract. To study the effects of time in culture on prolactin (PRL) and growth hormone (GH) responses to exogenous stimuli, bromocriptine (10^{-7} *M*) or somatostatin (10^{-7} *M*) were added to primary cultures of dispersed rat anterior pituitary cells (DC). Cells which had been in culture for 3, 6, or 9 days were then incubated for 6 hr and the media were radioimmunoassayed for PRL and GH. The inhibitory effects of bromocriptine on PRL release (76% decrease) and somatostatin on GH release (62% decrease) from cultured cells were maximal on Day 3 and decreased with age of the culture. In addition, the inhibitory effect of bromocriptine on GH release (32% decrease) was slight but maximal on Day 3 and decreased with time in culture. In contrast, somatostatin showed slight and stable inhibition of PRL release (26 to 29% decrease) at each of the three time periods. At the end of each incubation, the media were replaced with Ham's F-10 medium lacking bromocriptine or somatostatin and the cultures were incubated again for 6 hr. Even after removal of these agents, the inhibitory effects of bromocriptine on PRL or somatostatin on GH release persisted at almost the same or a higher degree compared to the controls. On the other hand, the inhibitory effects of bromocriptine on GH or somatostatin on PRL disappeared almost completely after removal of the agents. The release of both PRL and GH into the media was greater in all cases, including the controls, during the second incubation. This may have been due in part to the stimulation caused by changing the media. The results suggest: (1) rat pituitary mammotrophs and somatotrophs may both possess receptors for bromocriptine and somatostatin (2) in the absence of hypothalamic control (i.e., *in vitro* system), these cells are most sensitive to both agents on Day 3, and (3) the rebound increases seen *in vivo* following these agents are likely caused by hypothalamic modulations.

The effect of the age of primary cultures on the course of basal hormone secretion and on the reponsiveness of anterior pituitary cells to exogenous stimuli has not been well studied. In addition, the mechanism of rebound increases in prolactin (PRL) and growth hormone (GH) secretion in rats and humans after cessation of treatment with inhibitory agents is not fully understood (1–7). To investigate these important questions, we used primary cultures of rat anterior pituitary cells and examined the basal secretion and the responsiveness of the mammotrophs and somatotrophs to bromocriptine and somatostatin on Days 3, 6, and 9 in culture.

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Materials and Methods. *Dispersed cell cultures.* Male Sprague–Dawley rats (Charles River Corp., Wilmington, Mass.), weighing 200–250 g, were housed in an artificially illuminated (14 hr light, 10 hr dark) animal room for periods of 1 to 2 weeks before sacrifice. Food and water were available *ad libitum*. Twenty-three animals were killed at 09:00 hr by decapitation. The pituitary glands were removed aseptically under a laminar flow hood, the posterior lobes were discarded, and the remaining tissue was placed into a sterile petri dish (5-cm diameter) and minced to the size of 1 mm^3 in Hank's balanced salt solution without Ca^{2+} or Mg^{2+}. The minced pituitary tissue was washed gently with Hank's solution five times to remove blood cells. Then 5 ml of 0.1% trypsin (Grand Island Biological Co., GIBCO, Grand Island, N.Y.), solubilized in Hank's solution containing 0.1%

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bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, Mo.) was added to the petri dish, and the tissue was then transferred to a 35-ml Erlenmeyer flask. The solution was agitated gently by a stirrer for 10 min, and the supernatant, which contained dispersed pituitary cells (DC), was collected by filtration through lens paper (Matherson Scientific, New York) in an ice-cooled 50-ml Erlenmeyer flask. Then, 5 ml of Ham's F-10 medium supplemented with 10% fetal calf serum and 2.5% horse serum was added to the flask to block the action of the trypsin. The trypsinization and cell collection procedures were repeated five times. Finally, the cell suspensions were centrifuged for 5 min at 1000 rpm, and the cells were resuspended in Ham's F-10 medium. These cells were cultured in T-25 flasks (Corning, No. 25100) at 37° for 3, 6, or 9 days in an atmosphere of 5% CO_2 and 95% air. The number of cells per flask was 16.5×10^4, and the cell viability, as determined by the trypan blue dye exclusion method, was over 95% before and after 9 days of cell culture.

Effects of somatostatin or bromocriptine on PRL and GH release. After 3, 6, or 9 days of cell culture, the medium was discarded and 10^{-7} *M* of somatostatin (GH release inhibiting hormone; GH-IH, Sigma) or 10^{-7} *M* of bromocriptine (2-Br-α-ergocriptine; CB-154, Sandoz) dissolved in Ham's F-10 medium lacking serum (pH 7.7) was added to the DC cultures. Cells cultured in serum-free Ham's F-10 medium were used as controls. Five flasks were used for the control cultures or for the 10^{-7} *M* concentration of each agent, respectively. After 6 hr in the presence of these agents, the media were collected. These samples were kept frozen at −27° until assayed.

PRL and GH secretory responses after the removal of the agents. After the collection of the media mentioned above, 5 ml of Ham's F-10 medium lacking serum and agents were added to every flask, and the cells were again incubated for 6 hr to see if rebound increases in the hormones released would occur *in vitro*. The media were then collected and kept frozen until assay. In both experiments, the effects produced by the agents were compared to the results obtained in control cultures.

RIA of rat PRL and GH. Rat PRL was measured in duplicate by radioimmunoassay (RIA) methods previously described (8). Rat GH was also measured by double antibody RIA procedures using materials supplied by the NIAMDD. All samples were assayed in duplicate according to the procedures recommended by NIAMDD. Results were expressed in terms of the NIH standards, PRL-RP-1 and GH-RP-1. Intra- and interassay coefficients of variation were 2.1 and 4.8% for PRL, and 5.9 and 7.5% for GH, respectively.

Statistical analysis. The data were subjected to analysis of variance followed by the Student–Newman–Keul's test for comparing the differences between group means.

Results. *Effects of somatostatin or bromocriptine on PRL release.* The basal secretion rate of PRL increased progressively with increasing time in culture (Fig. 1A). At each of the three time periods not only bromocriptine but also somatostatin significantly suppressed the rate of PRL release compared to the control values ($P < 0.01$; Fig. 1A). When these data were expressed as a percentage of the control value (Fig. 1B) the inhibitory effect of somatostatin on PRL release was constant and varied from 26 to 29% at the three time periods. In contrast, the inhibitory effect of bromocriptine on PRL release was maximal on Day 3 (76% decrease from basal), and became attenuated at 6 days and at 9 days (52 and 32% decreases in PRL release from basal on Days 6 and 9, respectively). The inhibition by bromocriptine was far greater than that produced by somatostatin on Day 3 ($P < 0.01$) and on Day 6 ($P < 0.01$), but not on Day 9 (Fig. 1A).

PRL secretory responses after removal of the agents. After removal of the media containing the inhibitory agents the DC were reincubated for 6 hr in Ham's F-10 medium alone. Again the control flasks showed increases in PRL release with increasing time in culture and each value exceeded that of the former incubation (Fig.

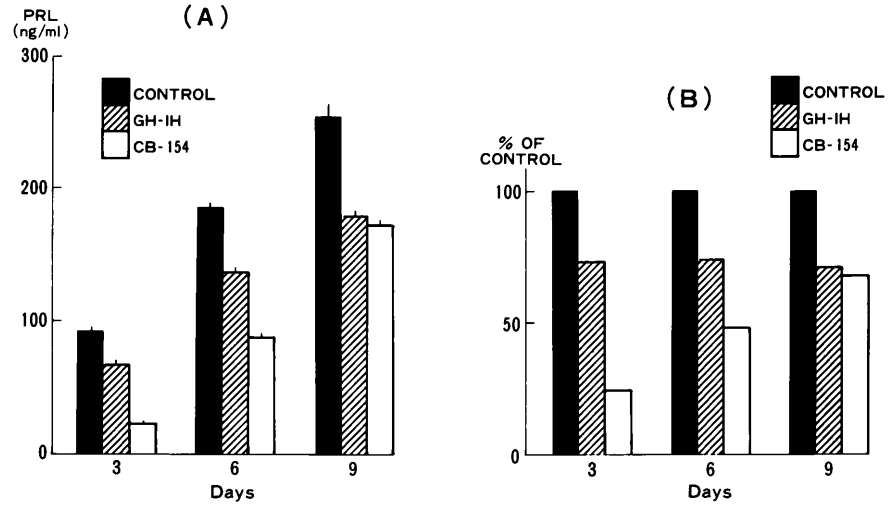

FIG. 1. (A) Effects of somatostatin (GH-IH) and bromocriptine (CB-154) on the release of PRL into Ham's F-10 culture medium during a 6-hr incubation. Each bar represents the mean ± SEM. (B) The mean values were expressed as percentage of control (100%).

2A). Even after the removal of the bromocriptine from the cultures the PRL inhibitory effect of this agent continued at almost the same degree as was seen with bromocriptine in the medium (71, 56, and 52% decrease from basal on Days 3, 6 and 9, respectively; $P < 0.01$ compared to control). In contrast, the removal of somatostatin from the media largely freed the cells from its inhibitory effect; only in the 9-day cultures was there still a significant inhibition of PRL release as compared to the control group ($P < 0.01$; Figs. 2A, B).

Effects of somatostatin or bromocriptine on GH release. Basal GH release rate increased with time in culture and reached a

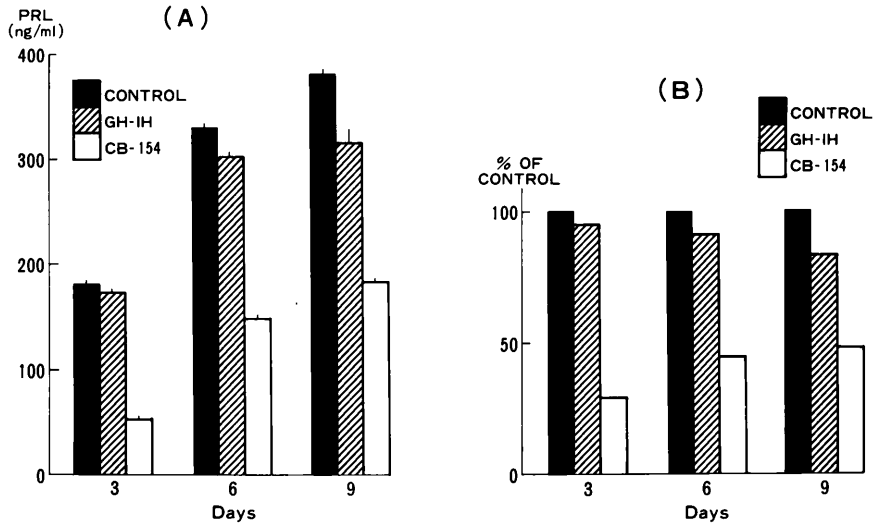

FIG. 2. (A) PRL secretory responses during 6-hr incubation with agent-free Ham's F-10 culture medium after the removal of somatostatin (GH-IH) and bromocriptine (CB-154). (B) See legend of Fig. 1B.

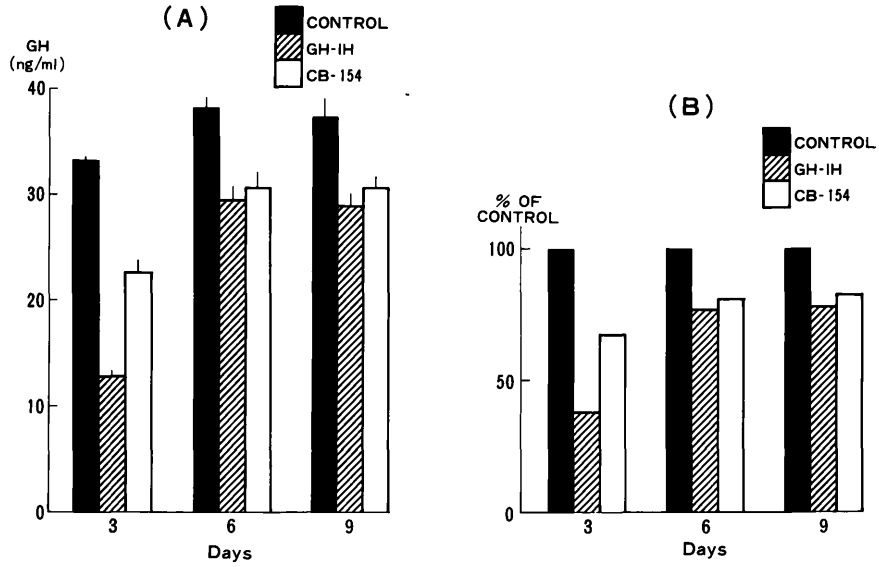

FIG. 3. (A) Effects of somatostatin (GH-IH) and bromocriptine (CB-154) on the release of GH into Ham's F-10 culture medium during a 6-hr incubation. (B) See legend of Fig. 1B.

plateau at 6 days (Fig. 3A). At each time, somatostatin and bromocriptine significantly inhibited the GH release compared to controls ($P < 0.05-0.01$). As shown in Fig. 3B, the inhibitory effects of somatostatin or bromocriptine on GH release were maximal on Day 3 (62 and 32% decrease, respectively) and were diminished on Day 6 (23 and 20% decrease) and on Day 9 (22 and 17% decrease, respectively). On Day 3, the inhibitory action of somatostatin was far greater than that of bromocriptine ($P < 0.01$). However, there were no significant differences between the inhibitory effects of somatostatin and bromocriptine on Days 6 or 9.

GH secretory responses after the removal of the agents. After removal of the inhibitory agents, the GH released in the control group was maximal on Day 3, and lessened with time although all of the basal control values were far greater than those seen in the first incubation (Fig. 4A). As with the PRL responses to bromocriptine, after the removal of somatostatin, the inhibitory effect on GH release persisted at a slightly higher level than that seen during the first incubation (67, 50, and 49% decrease on Days 3, 6, 9, respectively, $P <$ 0.01 compared to control; Figs. 4A, B). On the other hand, the cultures that had been treated with bromocriptine were freed from inhibition and showed no values which differed significantly from the controls (Figs. 4A, B).

Discussion. This study showed that bromocriptine or somatostatin can markedly suppress the rate of PRL and GH release from primary cultures of dispersed rat anterior pituitary cells. With increasing time in culture the PRL- or GH-inhibiting activity of bromocriptine or somatostatin, respectively, became somewhat decreased. Moreover, we observed no rebound increases in the release of either hormone after the removal of these inhibitory agents.

It has been reported that dopamine (DA) or DA agonists can suppress PRL and GH secretion in adult rats (9–15), although DA may have a stimulatory role on GH secretion in infant rats (16). In this study, bromocriptine had an inhibitory effect not only on PRL but also on GH release *in vitro*. Thus bromocriptine acts on pituitary mammotrophs and somatotrophs directly. Therefore, it seems possible that rat pituitary mammotrophs and somatotrophs both have receptors for bromocriptine. It has

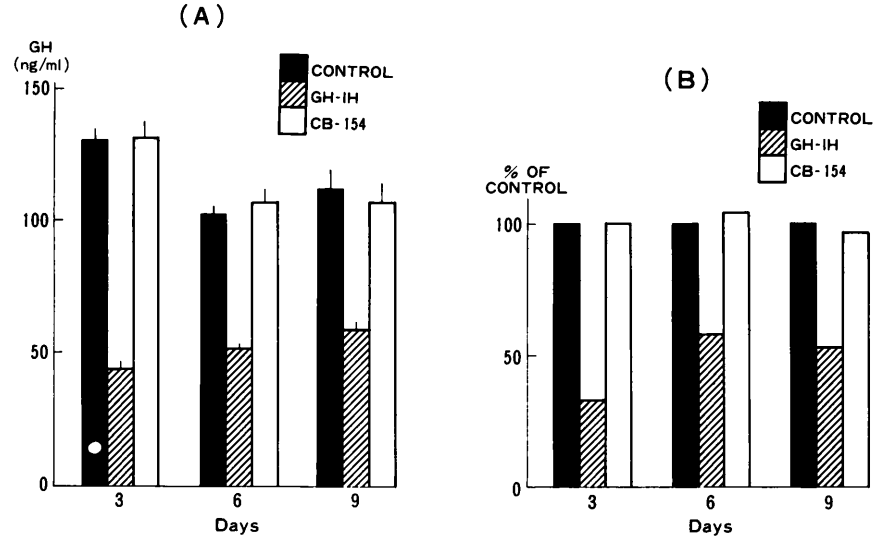

FIG. 4. (A) GH secretory responses during 6-hr incubation with agent-free Ham's F-10 culture medium after the removal of somatostatin (GH-IH) and bromocriptine (CB-154). (B) See legend of Fig. 1B.

been demonstrated that receptors for DA and apomorphine are both present in cells of the pituitary gland (17–19).

In rats, somatostatin suppresses GH secretion both *in vivo* and *in vitro*, while it inhibits PRL secretion only *in vitro* (20–22). Our results confirmed the *in vitro* effects of somatostatin on GH and PRL release. The reason somatostatin inhibits PRL release only *in vitro* is not known. One possibility may be that somatostatin can reveal this action only in the absence of hypothalamic regulation. In normal human subjects, somatostatin does not inhibit basal or TRH-induced PRL secretion (23, 24). However, it inhibits the basal levels of PRL in some acromegalic patients (25) and arginine-induced PRL release in normal men (26). In acromegalic patients, the properties of mammotrophs and the hypothalamic function of the tuberoinfundibular dopaminergic neurons may be different than in normal subjects (25, 27, 28).

Studies on the time course of the responsiveness of primary cultures of dispersed anterior pituitary cells to bromocriptine or somatostatin have not been previously reported. Bromocriptine had maximal inhibiting activites on PRL or GH release on Day 3, and this activity became lessened with time. Somatostatin caused a maximal inhibition of GH release on Day 3, and this inhibition decreased with time. In contrast, somatostatin showed a slight but stable inhibition of PRL release at each of the three times tested. Why bromocriptine and somatostatin had maximal inhibitory activities on PRL or GH release on Day 3, respectively, and then showed a lesser activity with increasing time in culture is not clear. These findings are at variance with the reports that surgical or pharmacological disruption of the hypothalamus causes supersensitivities of mammotrophs or somatotrophs to exogenous stimuli (29–31). However, in these experiments the pituitary glands were already manipulated *in vivo*, and the *in vitro* studies employed hemipituitary glands obtained immediately after decapitation. Therefore, the experimental conditions were quite different from ours and it is difficult to compare the results. Without the presence of receptor binding substances, it is possible that the number of receptors to regulating hormones and responsiveness of the cells will decrease, as has been suggested for patients with Addison's disease where

the high ACTH levels are not suppressed to normal ranges by glucocorticoids (32, 33).

Somatostatin and DA are important although not the only physiological regulators of GH and PRL secretion, respectively (9, 13, 20, 34). Bromocriptine is a potent DA agonist, and has been widely used as a substance to inhibit PRL release (13, 35). Although, the responsiveness of GH to somatostatin or PRL to bromocriptine changed markedly with time in our experiments those of GH to bromocriptine or PRL to somatostatin showed only slight changes. These findings may also suggest that, in the absence of hypothalamic control, somatotrophs and mammotrophs may lose their responsiveness to their major inhibiting agents, while the cells may retain their responsiveness to other less specific agents.

In vivo studies have shown that after the removal of inhibiting agents, PRL or GH are apt to show rebound increases exceeding the initial basal levels (1–7). We did not observe any rebound phenomenon in PRL or GH release after the removal of the inhibitory agents, although the level of hormones released from all the cultures, including the controls, was higher after the removal of somatostatin or bromocriptine. We believe these increases as well as the higher basal levels of hormone release must have been due to at least in part to the stimulatory effect of changing the culture media (36, 37).

There are several possible explanations for the mechanism of the post-inhibitory rebound seen *in vivo*. The rebound could be due to (1) increased hypothalamic releasing factors; (2) decreased hypothalamic inhibiting factors; or (3) an overshoot from the stored pituitary pool (3, 5). Our results mainly support the first or second explanations because without the hypothalamic control *in vitro* there were no rebound phenomena. These findings are similar to those of Martin *et al.* in which hypothalamic VMN (ventromedial nucleus) lesions in rats prevented the somatostatin-induced postinhibitory rebound (3).

In conclusion, rat pituitary mammotrophs and somatotrophs have been shown to respond *in vitro* to both bromocriptine and somatostatin. With the removal of hypothalamic control (i.e., *in vitro* system), these cells were most sensitive to these inhibitory agents on Day 3 in culture. Also, it is proposed that the post-treatment rebound increases exceeding basal levels in PRL and GH secretion seen *in vivo* must be mainly caused by hypothalamic modulations.

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